GREGOR JOHANN MENDEL
(1822—1884)

GENETICS

BANGALORE
1938

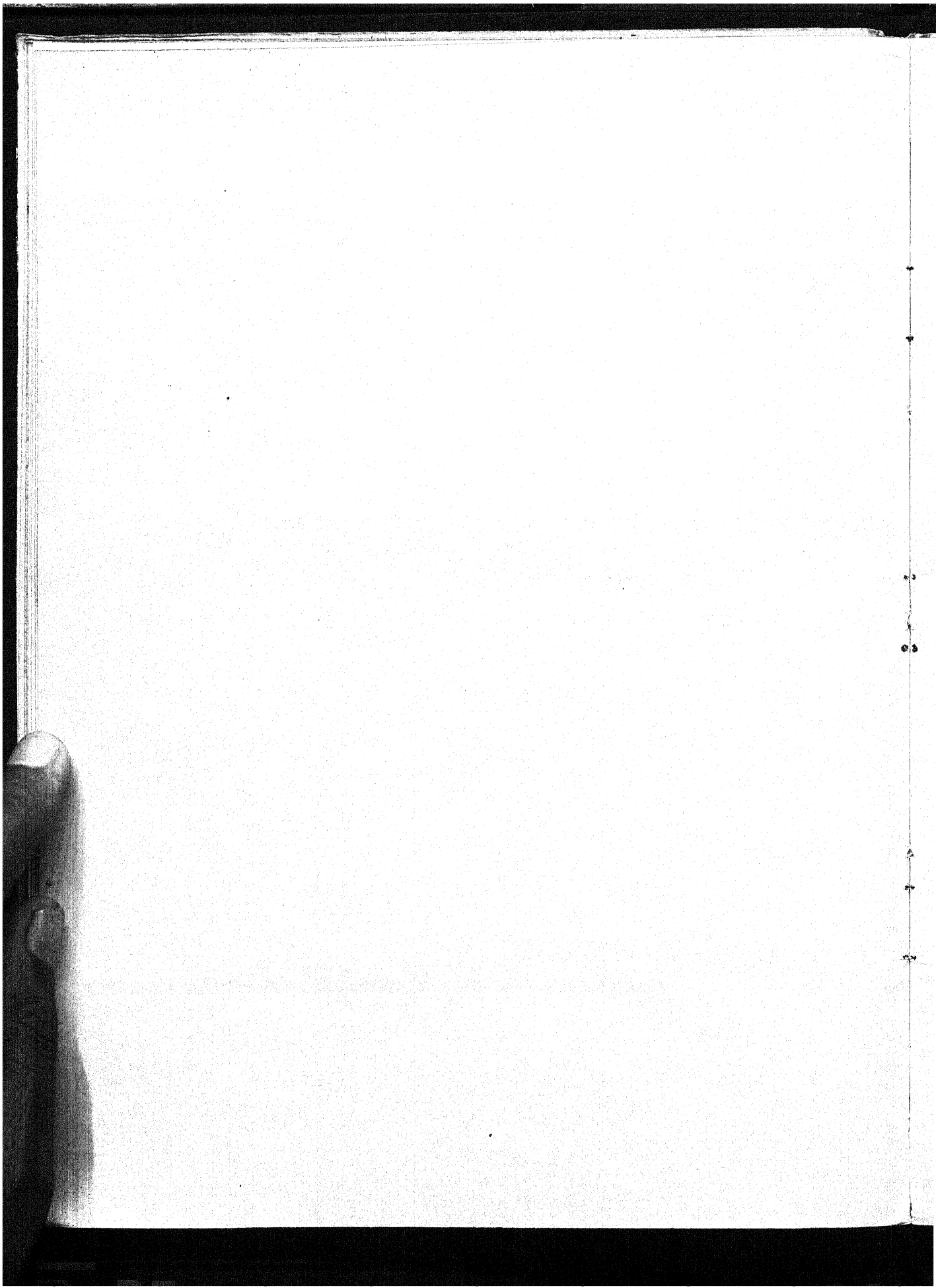
PREFACE.

THE third of the series of supplements organised by *Current Science*, embodying authoritative exposition by the leading scientists on latest advances in the different branches of knowledge, is now issued. This supplement deals with the various aspects of the study of Genetics, and we have no doubt that this number will be received by our readers and the general public interested in scientific problems with the same generous enthusiasm, accorded to the first two numbers on *Laue Diagrams* and *Canal Rays*. We have pleasure in acknowledging our deep sense of gratitude to Dr. Eileen W. E. Macfarlane for the arrangement of matter for the press and for the general introduction contributed by her. We are also thankful to Mr. B. R. Seshachar for assisting us in reading the proofs.

We would like to take this opportunity of announcing that the fourth supplement dealing with *Organisers in Animal Development* is now in the Press, and that the special articles have been contributed by Dr. O. Mangold, Dr. Eckhard Rotmann, Prof. Dr. von Johannes Holtfreter, Dr. Paul Weiss, Dr. Wolfgang Luther, Dr. C. H. Waddington, Prof. Sven Horstadius, Dr. C. M. Child. This supplement is profusely illustrated, and we hope that the Biological Departments in the universities would seize the opportunity of possessing copies of this superb treatise.

Editor.

CONTENTS.



GENETICS

INTRODUCTION.

By Eileen W. Erlanson Macfarlane, *Calcutta.*

(*Received 11th November 1937.*)

GENETICS has not received its fair share of attention from Indian scientists. Because we have done relatively little to advance their subject, we are all the more beholden to those who have contributed to this Special Number.

The founder of Genetics, the Austrian monk Gregor Johann Mendel, was a physicist. In his hobby of plant breeding, he discovered some of the chief laws of heredity and published them in 1865. His work was ignored by the scientific world until these laws were rediscovered by three people independently in 1900. Therefore this important branch of biology is actually under forty years old. In 1909, Bateson published "*Mendel's Principles of Heredity*" and demonstrated that Mendel's laws held good for many plants and animals. About this time nuclear meiosis was recognised as providing the necessary mechanism for Mendelism. Thus, very early, genetics reconciled Botany and Zoology, and also gave the struggling cytologist a place of honour. Apart from the ignorance and superstition that this study has destroyed, it has ushered in a pleasant era of co-operation between workers in all fields of biology. Everyone who visits the great Genetics laboratories in America will be impressed by the fine spirit of mutual aid that exists between the scientists and their students both in the same and in different institutions. Anyone who is genuinely interested may obtain carefully bred stocks of Drosophila or Zea Mays for the asking, and he may be provided with unpublished data on them to boot. If a Botanist chooses to breed fishes or a zoologist to grow Evening Primroses there is no question of poaching.

Cytology has elucidated genetic data and makes it comprehensible to any layman by the pictorial method.

After the establishment of Mendel's principles the greatest advance came from Columbia University, U. S. A., where Morgan, Muller, Bridges and Sturtevant made an intensive study of the fruit fly—*Drosophila melanogaster.* These men were the first to observe point mutation and to locate the Mendelian factors or genes in the chromosomes and we are very fortunate to have contributions from two of this famous tetrad. Chromosome maps now exist for several species of Drosophila, for Zea Mays (Emerson and colleagues), for Lathyrus (Punnett) and for Pharbites (Imai). A beginning has been made in mapping the X-chromosome in man (Haldane). Professor Muller's article on the gene and Dr. Bridge's article on the future of genetics will interest readers all the more because they are among the foremost authorities on the subject. Dr. Muller became world famous in 1927 when he announced his discovery that mutation rate can be increased many times by the use of X-rays. For the past four years he has been engaged in genetical work in Moscow, U.S.S.R.

The phenomenon of mutation, the sudden appearance of a new transmittable characterstic, was first recorded in the Evening Primrose (Œnothera). These mutations of De Vries were caused by changes in the chromosome set and not in single genes. Oenothera possesses an anomalous type of hereditary mechanism, and it took the combined efforts of a dozen or more people all over the world some twenty years to prove that the exceptions to Mendelism, exhibited by many of the species, were only apparent and due to a complex type of heterozygosis.

Important contributions to our understanding of sex-determination have been made by genetics and cytology. Professor Winge was a pioneer in such work among vertebrates. His material, the little tropical fish Lebistes, is beautiful and admirably suited to the purpose.

The species problem, which has long been an absorbing obsession with biologists has been considerably elucidated by experimental taxonomy which involves genetics and cytology. Vavilov found that the parallel

variations that exist in related species are usually inherited in a similar manner. Differences between some species have been shown to be due chiefly to one or more pairs of allelomorphic genes; and between others to lie in the structure or number of the chromosomes. Many cultivated plants have been shown to be amphidiploid hybrids. The Linnean species *Galeopsis tetrahit* was synthesised by Müntzing through the hybridization of two diploid species, followed by a fortuitous chromosome duplication in the sterile offspring. The cytological aspects of interspecific crosses among polyploid series have been carefully investigated for many years by Professor Kihara and others. Kihara and Ono first distinguished between autopolyploids and allopolyploids with several unlike sets of chromosomes from different species. We have also gained an understanding of the genetics of polyploids. One of the outstanding pieces of research in cytogenetics and experimental taxonomy is that of Professor Babcock and his co-workers in California on the large genus Crepis. This project is of epic proportions and is a valuable exposition of the processes of species formation and evolution in relation to geographic distribution, morphology and cytology.

With his characteristic vigour and clarity of thought and exposition Professor Shull has evoked much interest among biologists recently by tackling the classical myth of adaptation from the genetical standpoint. It should gratify the older biologists to know that the theories of their *guru*, Charles Darwin have been largely ratified in recent years by genetics. Our present theory of evolution is a slightly modified Darwinism.

Human genetics still consists chiefly of collections of pedigrees. One significant fact that has been brought out is the variable potency of some mutant genes which are dominant in some stocks and recessive in others. Genic effect is not as straightforward as it used to be supposed, and in people each gene may be influenced to some degree by all the other genes present. The multiple allelomorphic genes which are responsible for blood groups, discovered by Landsteiner and Bernstein, are among the few clear-cut cases of simple Mendelian inheritance in man. More complete data have exonerated blue-eyed parents who have had a brown-eyed child, for eye colour is now found to be affected by modifying genes and is not always a simple Mendelian character. For the exposition that human skin colour and height are consistent with the influence of multiple factors, we are indebted to Davenport and Fisher respectively. Dr. Davenport is the leading eugenicist in America and his paper on human hybrids should stimulate wide interest in India. Although the application of statistical methods has shown interesting possibilities, human genetics and population problems are difficult fields in which a beginning has scarcely yet been made. It is of the utmost importance to curb our enthusiasm for practical application and to do all in our power to prevent gullible or crafty politicians from seizing upon our humble findings as sticks with which to beat some innocent sections of the human family. The present-day faith in science may produce effects as pathetic as those of pre-historic totemistic beliefs unless the public are made to understand that all scientific conclusions are tentative, and that every scientist looks forward to having them improved upon.

In our every-day life genetics has opened up vast prospects in the improvement of domestic plants and animals an aspect with which Dr. Frost's paper deals. Genetics has destroyed several age-old superstitions. If a desired characteristic such as disease-resistence or hardiness, is present in some members of a genus, then in all probability it can be incorporated in some other more useful member in which it is absent. Greatly increased yields in seed, foliage and sugar content have been obtained. The phenomenon of sex-linkage has been utilized to determine the sex of chickens at the time of hatching, and horse and cattle breeders have been taught how to eliminate lethal genes. Bulls are now graded according to the milk-yield of their daughters. No longer need a valuable pedigreed female be destroyed if she be accidentally crossed by a mongrel or male of another breed. Genetics shows that previous mates cannot effect subsequent offspring by other males, thus removing whatever prejudice may have existed against widow remarriage.

Sex-determination has been explained and shown to be beyond the control of either parent but directly due to the male gametes. Normally, sex is fixed once and for all at conception. Among humans the male has been proved to be the "weaker", more delicate sex, and the female to be more fit

biologically and to possess greater vigour. The difference between the sexes is found to be merely quantitative. Eventually, it may be possible to combine the desirable characteristics of both. The old wives' tale of parental influence or so-called "maternal impressions", has been discredited. Pregnant women need not fear the effects of alarming sights nor need they bother to study art nor mathematics hoping that the child will thereby become beautiful or clever. We know that inbreeding only brings to light weaknesses already existing, instead of creating them; likewise it may increase strength in the best stocks.

Since behaviour and character are dependent upon the environment and are not transmitted, the old adage "blood will tell" is fallacious.

Alone among modern scientists geneticists have not yet won their right to free speech for their working hypotheses and conclusions. Genetics has already shown that all men are cross-breeds of various degrees and has thus removed the main prop of the antiquated theory of discrete races among us. Just as Darwin, Wallace, Weismann and other naturalists of the last century destroyed the theory of special creation of species, so modern geneticists have shown that both race and nation are dynamic, continuously mutable aggregations. These conclusions are gradually penetrating the mind of the public, and the effects are seen in the death struggles of nationalism which is destroying itself and which will pull down imperialism in its demise.

Authorities no longer mind the earth being round, nor the fact that the physicists think that matter is "a wave of probability undulating in nothingness". But a humble geneticist may get into serious trouble if he ventures to say that his data indicate that one human type is, or is not, more fit than another. Emotions are out of place in Science, for scientifically speaking there is no such thing as better or worse, there are only differences. The physicist has destroyed our classical concepts of time and space, perhaps genetics will destroy the concept of personality as something separate and autonomous.

Although our pleasurable anticipation of an International Genetics Congress has been deferred, it is hoped that this Special Number will arouse a wider interest in the subject in India. Because our field has not the extreme abstractness of physics it is less exact and more easily understood. Most of these articles can be assimilated by any cultured person. May they arouse educated thought in this country to realize the importance of genetics to economics (plant and animal improvement) and to sociology, as well as to the vast scope for genetical studies here.

THE PRESENT STATUS OF THE MUTATION THEORY.[1]

By H. J. Muller.

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(*Received 7th January 1937.*)

WHAT the quantum theory is to modern physics, the mutation theory is to modern genetics; for mutations provide the fundamental units of change lying at the basis of all genetic differences, including even the grand differences between distant evolutionary divisions, even as quanta lie at the bottom of all greater differences of energy content. Moreover, as in the case of quantum changes, so too in the case of mutations, the changes are sudden and discrete, and are punctuated by interphases of stability, often of a very high order. We owe it to de Vries to have definitely set us on the path of this quantum theory of biology. Unfortunately, however, de Vries soon came to deal mainly with a form, Oenothera, in which the processes of genetics are maximally intricate, and which therefore provided an unsuitable basis for the elucidation of the underlying priciples that apply to the primary processes of mutation. In what follows, however, I shall not attempt rigorously to separate the earlier from the latter contributions, but, following the logical rather than the chronological order, shall try to present the mutation theory in the guise which it appears to me to take at present. For this purpose, it will of course be necessary to review much that is to-day well known and accepted.

The Gene as the Material Basis of Mutation.

Fundamental to an adequate conception of mutation is a valid conception of the gene—the material unit of heredity in which the primary mutations occur. The whole drama of life and evolution is a resultant of the conflicting play of forces that grow out of the gene's peculiarly opposite properties of multiplying itself unchanged, and of mutating. These properties do not annihilate each other nor come into a permanent mechanical equilibrium, precisely because when the gene varies (mutates), its property of self-multiplication is not destroyed, but becomes transformed correspondingly, so that it now reproduces its *new* type, and, if thereafter successful in its competition for existence, the same cycle of process is now repeated from the new basis. It is by virtue of this kind of progressive cyclical activity then that the gene serves as the basis of all biological evolution, and hence of the most essential distinction between lifeless and living matter. That is, the gene is the basis of life, not merely because it can reduplicate itself exactly, but just as much because it is so constituted that it undergoes change (mutation), without losing the power of further reduplication, even of this very change itself.

It remains for the physical-chemical science of the future to find out how this extraordinary combination of properties results from the physical and chemical structure of the gene molecules working in relation with their surroundings. As time goes on, various newer findings have rendered more probable the earlier inference, which Koltzoff among others has insisted upon, that the genes are of protein nature, and that the geno-chemist must therefore work in this field.[2] Gene multiplication, when regarded analytically, is, of course, not some sort of swelling and "division," but consists of an actual building up of a new gene by the side of the old and in the image of the old gene itself. Although this process comes under the chemists' conception of autocatalysis, as first recognized by Jacques Loeb for the chromatin as a whole and later by Hagedoorn and by Troland for the individual genes, it is a highly peculiar type of autocatalysis, not readily explained by consideration of already known principles applicable to other chemical types. The peculiar specific auto-attraction of like genes for one another is, I believe, a related property,

[1] Presented at de Vries Memorial Meetings of the Institute of Genetics, in Moscow, and of the Leningrad Society of Naturalists, in Leningrad, November 1935.

[2] Recent work of Stanley shows that the mosaic viruses of tobacco and of tomato may be obtained as crystalline proteins. Since these bodies are self-reproducing and at the same time capable of mutation, they have the essential qualifications for being regarded as genes. This is a virtual proof of the protein character of genes, and offers the remarkable opportunity of studying the properties of genes by means of direct physico-chemical investigation. It is interesting to note the approximate correspondence of the size of these molecules with our provisional estimates for gene size in Drosophila.

particularly since the conclusion cannot be escaped that the mutation of a gene likewise changes its attractive (synaptic) property in just such a way as still to leave it auto-attractive.

In the endeavour to form a more adequate conception of the nature of the gene and of its mutation, the two interconnected questions concerning the number of genes and the size of the individual gene must be of considerable significance. There was already evidence 15 to 20 years ago for the conclusions that in Drosophila there must be some hundreds of genes, at least, in the X-chromosome alone, and that the individual gene must be of submicroscopic size (Muller, 1916, 1922; Muller and Altenberg, 1919). These conclusions were arrived at by means of two different methods of arithmetical reasoning utilizing as data the frequency of recurrent mutation, and of crossing over, respectively. These methods involved considerable elements of uncertainty and error.[3] Fortunately, however, we now have a new method: that of studying the maximum possible number of chromosome breaks between detectible genes that can be produced by X-rays within a given limited chromosome region, a region whose total physical length can be directly measured in the salivary gland chromosome (Muller, and Prokofyeva, 1934, 1935; Muller, 1935). This method has again shown the "gene" to be of submicroscopic volume. Although its length (or at least the distance between the end of one gene having a detectible effect and that of the next one) seems to be just at the borderland of the microscopic, its thickness cannot be more than a fifth of the probable value for the length and may be much less than this. Unless, then, the detectible genes are separated from one another by non-genic material, or by genes having no effect that we could detect, their shape must be fibre-like, as was to be expected of a large organic molecule, such as that of protein.

The sudden and discrete character of gene changes, and the long interphases of stability, suggested practically from the first that these probably were chemical changes, so that indeed the genetic quantum theory might really be regarded as a not very remote expression in genetics of the quantum theory of physics itself. This would be the more true if the individual gene was a single molecule (or "super-molecule") and if the mutations were isolated molecular changes. That the gene should be regarded as a single molecule, or at any rate as not composed, like grosser bodies, of a group of like molecules, has been shown by the fact that, when a mutated gene reproduces itself it shows immediate stability. That is, there is no sorting out, in subsequent cell-generations, of mutated from non-mutated molecules, for no crazy-quilt mosaic pattern of abnormal somatic tissue is found in either the soma or germ cells of the individuals containing a newly mutated gene. This reasoning involves, to be sure, the notion that the different molecules would probably mutate individually, in isolated fashion. Now the fact of the comparatively isolated character of the mutational events was early evident in organisms like Drosophila, having comparatively uncomplicated genetics, for cases of simultaneous mutation of two or more different genes were rather rarely found, and it could further be inferred from certain peculiarities of the data that even two identical genes lying in close proximity with one another in the same cell ordinarily mutated independently of one another. From this the conclusion was to be drawn that mutations have the nature of microchemical accidents: "When the molecular or atomic motions chance to take a particular form, to which the gene is vulnerable, then the mutation occurs (Muller, 1920).

On this conception, then, the gene is a

[3] These methods and results, which had meanwhile been cited by Morgan and his co-workers (1922, 1925), were not until later given in detail (Muller, 1926). Unfortunately, however, the calculation that was based upon recurrent mutations involved the neglect of an important term—the factorial in the denominator of the Poisson series representing the frequency of mutations occurring 0, 1, 2, 3, etc., times. Wright was kind enough to direct my attention to this error some years later. The inclusion of the factorial would have somewhat more than doubled the final value of gene size obtained by this method, and approximately halved the gene number. This would still have left the final results of the same order of magnitude, and would not have altered the general conclusions, especially since the size estimate was to be regarded as only a remote upper limit, and the number estimate as a lower limit. More recently, for smaller estimates of gene size have been made by Gowen (1933). It must be pointed out that these are based upon this same method, including the above mistake, and that they make the further mistake of applying the method quite illegitimately. For in the latter data, unlike that which we utilized, there was absolutely no way of getting a valid estimate of how many of the supposed "mutations" (lethals produced by X-rays) were really recurrent—tests of allelomorphism not having been made; moreover, a large part of these "mutations" were really deletions and other chromosome abnormalities.

kind of molecule and ordinary mutations are to be regarded as the results of isolated micro-chemical accidents, not individually controllable. Such accidents would, of course, tend to occur with a given frequency under given conditions, just as, in ordinary chemical reactions, in which the gross change from one substance to another really is the statistical resultant of a whole series of individually accidental molecular collisions, the rate of change as a whole is fixed and calculable. In the latter cases we pay attention only to the effect *en masse* because the changes of the individual molecules are ordinarily too numerous, they come too close upon one another, and they are too small in their individual effects, to be considered separately. On the other hand, the genes in their medium are so much more stable than molecules undergoing chemical reactions in the test-tube, that is, they change so much more rarely, and each single change becomes, through the processes of reproduction and development, so magnified, that in this case the individual molecular changes rather than a mass effect usually constitute the objects of our investigation.

The Quantitative Study of the Normal Mutation Frequency.

Evidently, a more searching study of these matters required the development of quantitative methods whereby we were not dependent on the study of an isolated mutation here and there, but whereby the frequency, the direction, etc., of numbers of mutations could be determined under specified conditions. The pure line work of Johanssen and others following him had early shown that the individual gene must ordinarily have a very high stability, but this work could not be carried far enough to penetrate down to the level of frequency of mutational occurrences, and to arrive at any value for its order of magnitude. In somewhat later work on Drosophila, however, the requisite quantitative methods were worked out (Muller and Altenburg, 1919; Muller, 1922, 1928). The results obtained in 1918 and 1919 already showed that in this form gene stability is of the order of thousands of years, at a minimum figure, that is, not more than one mutation per individual gene would occur in this length of time. And in man, it was inferred, the stability must be much greater than in Drosophila, since the generation is so much longer that a time-rate of occurrence of lethals in man at all similar to that found in Drosophila would be genetically destructive in a single generation.

More detailed studies of a similar nature made on Drosophila since then show that, more usually, the value here is something of the order of 100,000 years (since ordinarily mutation occurs at a considerably lower rate than that originally found, and since the genes in which the observed mutations were taking place constituted samples from a total collection of genes considerably larger than the admittedly minimal estimate then used). Looking at the matter from the standpoint of mutation frequency rather than degree of stability, this means that only about one mutation ordinarily occurs in a given individual gene in Drosophila while it is being passed down through a course of about a million or more generations of individuals, or, conversely, but one mutation occurs in it in a lot comprising a million or more gametes produced simultaneously in a single generation. (Undetectable mutations, either too small to be seen or dominant lethals, are left out of account here.) This result agrees approximately with the value as since reckoned in the case of several sample individual loci in Drosophila, although there is no doubt considerable variation in the frequency in individual loci. Haldane and Penrose have recently reported on two loci in man which apparently give frequencies of the order of one mutation in 100,000 generations, that is, in about three million years. In maize Stadler reports values for individual loci ranging from one in thousands down to one in hundreds of thousands or even millions of years (the year here corresponding to the generation).*

It may be noted by the way that since each gene forms an image of itself some tens of times in each generation, the above rate of mutation for individual loci, even in Drosophila, means that not one detectible misstep is made in this image-formation in the course of several tens of millions of repetitions of it. That is, the copy of the copy of the copy—several tens of millions of times removed—is still sensibly identical

* There is, however, an extremely large error here which might have the effect of greatly lowering some of the above figures in maize. This is due to the unavoidable smallness of the number of cells serving as samples for the earlier stages of development—those stages which occupied by far the greater portion of the life-cycle.

with the original model. There is, moreover, reason to believe that, when the mutation does occur, it does not usually consist in a misstep in image-formation but in a change of the gene after its formation. For mutations are most easily produced in mature sperm cells, in which reduplication of genes must be at its lowest ebb.

Thermal and Chemical Influences on the Mutation Process.

On the view of gene mutation as a micro-chemical accident it was to have been expected that within the range of temperature normal to the organism, and thus not causing unusual chemical processes, a rise of 10° C. would increase the rate of change (that is, here, the frequency of mutation) several times, according to van t'Hoff's rule. Our quantitative results on mutation frequency in Drosophila soon gave evidence of this, and some preliminary experiments also suggested that perhaps at still higher temperatures, at which new and disturbing chemical processes might enter in, there might be a still greater acceleration of the mutation rate. Recent works of Timofeeff-Ressovsky have thoroughly confirmed the first proposition, concerning the effect of a rise in temperature within the normal range, and they have added to the probability of the latter, that the rise is more marked at abnormal ranges. But both his work and that of many others agree in showing that there is no such extreme and directive temperature effect as a few recent claimants, notably Jollos, have pictured.

In connection with the effect within the range of temperature normal to the organism, the physicist, Delbrück (1935), collaborating with Timofeeff-Ressovsky, has made a very important contribution. He points out that, as has been well known to physical chemists, the amount of increase in the rate of a reaction, caused by a rise of temperature, depends, according to a known formula, upon the rate of the reaction itself, at any one given temperature. If now we accept the previously presented view of the ordinary mutations as being due to micro-chemical accidents in the same sense as the changes of molecules in other chemical reactions are, and if we then take into account the rate of the mutation reaction of the gene at a given temperature, as determined by the data on mutation frequency, we find that, corresponding with the extremely slow rate of this reaction (many thousands of years intervening between one change of an individual gene molecule and the next), its rate of rise with temperature should be exceedingly high, as compared with the rate of rise of ordinary chemical reactions. The expected "Q_{10}" co-efficient thus calculated turns out, in fact, to have the unusual value of 6 to 8, instead of the ordinary value (which lies between 2 and 3).

Now the above-mentioned recent results of Timofeeff-Ressovsky on mutation frequency do in fact, as he points out, show a rise in the time-rate of mutation accompanying rise of temperature, that is considerably greater than that found in ordinary chemical reactions. The value actually found for Q_{10} is 5, and when the probable error is taken into consideration this is well within the range of Delbrück's figure. Re-examining my own and Altenburg's earlier results, derived from three separate sets of experiments, I find that all these agree in giving this high value for Q_{10}, when the time-factor is properly taken into account. Though in any one set of experiments the value is subject to considerable error, all of these experiments, earlier and later, taken together, can leave no doubt of the unusually high value of Q_{10}. We may conclude, then, that these correspondences of the observed and calculated values of Q_{10}, even where the values are so highly unusual, constitute a striking confirmation of the idea of gene mutation as being ordinarily a result of random inter- and intra-molecular motions. The randomness of course does not mean that they are fundamentally without law, but that they obey statistical rules, and that the exact mutation-determining configuration at any given time and point cannot be reckoned on the basis of merely general considerations of the nature of the gene, the cell and its surroundings.

If the above sketched chemical conception of the mutation process is correct, it might well be expected that abnormal chemical disturbances, if really occurring in the vicinity of genes, would not be without influence upon their rate of mutation, and so it is not surprising that abnormally high temperatures and some other conditions which probably entail drastic intra-cellular disturbances should result in a higher frequency of mutations. Thus, as Navashin has shown, the changes occurring towards the end of a period of aging in seeds lead to an increased

frequency of mutations (or at least of gene rearrangements). And Blakeslee finds that it is not so much the aging in itself which thus affects seeds, but the peculiar conditions of artificial preservation which may accompany it. In Drosophila some drastic chemical treatments that kill the organism as a whole have so far failed to give results, but Sacharov in Koltzoff's laboratory, and Lobashov in Leningrad University, have recently obtained results which indicate a probable influence of certain chemical treatments upon the mutation frequency: numbers more decisive than those yet published are still desirable here, however.

In none of these cases of drastic treatments, be it noted, has there been evidence of a directive effect. In view of the localized way in which mutations happen in only one allelomorph at a time, it should obviously be much easier to find a common disturbing factor that increases the likelihood of genic changes in general, than something so specific chemically as to act only in relation to the special peculiarities of structure of some particular gene, as distinguished from other genes. In the normal cell, however, all chemical disturbances are usually very efficiently excluded from penetrating as far as the gene, which is, as it were, the species' special trust to the individual. Ordinary physiological effects have not yet been found to reach and influence the mutation of the gene to an appreciable extent, except in the case of the special class of abnormal genes called "eversporting", in which the mutation frequency is thousands of times higher than the normal anyway. If effects of the same order of magnitude, relative to their own mutation frequency, were produced on normal genes, these could not be detected without experiments of great delicacy. And so there is no basis for the commonly held view of non-genetic biologists that not merely temperature, but in general, the conditions and habits of life to which organisms are subject must ordinarily have a great influence on the frequency of mutation within them. Still less can these conditions have a great influence on the general direction of mutation, for we must remember that phænotypic change in a given direction can be produced by the mutation of any one of many chemically different genes, all of which co-operate in the production of the character in question. Specific influences affecting one of these genes could not be expected similarly to affect the others, for they have nothing to distinguish them as a group from other genes except the fact that all happen to start reactions that influence the same final product. Least of all can we imagine that, through affecting the general direction of mutation, such conditions play an important part in determining the direction of evolution.

Certainly the conditions of life react in important ways upon the organism, and serve as the important counter-influences in determining the course of evolution through their effect *on selection*. But they are not the important factors in the determination of what mutations shall occur. The latter depend upon ultra-microscopic accidents whose nature is determined on the one hand by the inner peculiarities of structure of the individual gene itself, and on the other hand by the exact ultra-microscopic topography of impinging materials and forces that could not be predicted from a knowledge of the general exterior conditions. My meaning here may perhaps be better explained by saying that, given a practically homogeneous environment containing the means of subsistence, mutation and evolution would go on, though of course changes in environment often result in faster selection.

The Production of Mutations by Irradiation.

An influence upon chemical reactions which the cell is not able to exclude when this is applied to it is high-energy radiation. As has been proved by Altenburg (1930) in Drosophila and independently by Promptoff (1930) in Drosophila and by Stubbe (1930) in Antirrhinum and very recently confirmed and greatly added to by Stadler (1936) in maize, ultra-voilet light produces mutations. Since it activates molecules and thus directly and indirectly results in chemical reactions of many kinds, and since it is in fact selectively absorbed by chromatin, its effect on genes is readily understandable in terms of the general chemical idea of mutation above outlined. Here, however, we do not have a mere increase in the ordinary "heat motions" of all molecules, rendering a chance occurrence of mutations in the normal way more frequent, but a direct activation at particular points, independent of the heat motion that is present, but resulting in similar effects.

In the case of X-rays and rays of still greater energy content, likewise, there are

direct activations produced, at isolated points, and so these rays too produce mutations in large numbers (Muller, 1927; Stadler, 1928). In fact, these are the best known mutation-producing agents, for they penetrate much better than ultra-violet rays and hence can produce a given number of mutations before the surface becomes destructively burned, even when an entire organism or a mass of tissue is being treated. The frequency of mutations produced by these means, during the period of exposure to the radiation, can be raised to 50,000 or more times the ordinary spontaneous frequency. And by increasing the intensity of the rays, and shortening the period of exposure correspondingly, the increase in frequency in a given unit of time could, theoretically, be raised far beyond this, to an extent limited only by the power of the apparatus.

How direct the effect of X- or γ-rays usually is in causing the mutation of a gene we do not yet know, but the fact, several times confirmed—first by Oliver and by Hanson and, most recently by Raffel in our laboratory (Institute of Genetics, Moscow)—that the number of lethal mutations produced by X-rays is directly proportional to the dosage of the latter, indicates that the individual ionizations act independently of one another, and therefore rather locally, in producing mutations. This proportionality of the effect to the dosage is quite independent of the time-distribution of the treatment, and of the wave-length, as has been shown by a number of investigators—Hanson, Stadler, Patterson, etc.—and most decisively of all by Timofeef-Ressovsky. The lack of influence of wave-length proves decisively that the distribution of the ions produced is, within wide limits, of no importance, and that therefore, it is the individual ionizations which produce the mutations, when they happen to have occurred at an appropriate point. Agreeing with this conclusion is the further fact that the nature of the mutations produced—their locus, intensity and direction of effect—remains unaffected by changes in the amount or manner of distribution of treatment—only their frequency is dependent on the dosage.

Further evidence of the comparative directness of the X- and γ-ray effect lies in the findings that: (1) mutations are produced either within the cell-generation treated or not long after (Muller; Timofeef-Ressovsky); (2) that untreated chromatin entering at fertilization into the treated cytoplasm is not affected (as shown by the same authors); (3) that, as recently shown by Kerkis (in disproof of some contrary claims), the effect is not transmitted from one part of the body to another, and irradiation of only the gonad-containing end has as strong an influence as irradiation of the whole body.

But despite the above results, it has not yet been satisfactorily proved that the gene itself is always struck by an electron and ionized, in the production of a mutation, rather than affected secondarily as a result of some ionization nearby. In fact, there is some evidence in favour of the latter possibility, based upon the finding of several cases of simultaneous changes, involving visible characters, that affected two genes at once, in lots of material in which the numbers of visible mutations in single genes was low.* In one case of this general nature, reported by Panshin (1935), two genes, lying in sister chromosomes, were both affected, each being changed to a different allelomorph. If statistically significant, this work indicates some kind of spreading of the effect, from the original point of ionization, over a microscopic distance, a process which could only imply the occurrence of secondary reactions between the ionization and the mutation. In the production of gene rearrangements, especially, there is a reason to believe that intermediary reactions occur, for these rearrangements nearly always involve double or multiple breaks of the chromosome thread; and nevertheless they are not proportional, in their frequency, to as much as the square of the number of ionizations—as they should be if each one of the breaks was produced independently of every other break. (Unlike gene mutations they are however proportional to somewhat more than the number of ionizations themselves, as mentioned in a subsequent section.) This again indicates that two or

* These may in part have involved chromosome breakage and rearrangement, rather than mutation within the gene, but the mutation or breakage at one point had no apparent connection with that at the other point in question. For instance, in one case there was an inversion in the right part of the X chromosome resulting in Bar eye by its "position effect," and at the same time there was a reversion of the gene for vermilion eye to its normal allele, though this lay in a chromosome region that had retained its normal arrangement of genes.

more of the events in question (here, chromosome breaks) depend upon one ionization, by some secondary " spreading " process.

Whatever may be the answer to the question of the degree of directness of the effect, the fact must also be noted that the condition of the X-rayed cell influences the ease with which mutations can be produced by X-rays in that cell. On the one hand, anæsthesia certainly, and general metabolic activity probably, have little if any effect of this sort (see Hanson, Kossikov, Serebrovskaya, and also unpublished work of Offermann). But some other differences in cellular conditions do exert a distinct influence. Thus, in Drosophila, mature sperm are much more readily affected by X-rays (so as to produce mutations), and mature eggs somewhat more readily, than immature germ cells. [In reaching this conclusion regarding the differential X-ray mutation rate of germ cells in different stages, we have not confused it with the effect due to the differential multiplication rate of mutated and non-mutated cells in the gonads—a hitherto questionable phenomenon the reality of which has recently (1935–36) been proved in data independently obtained by Kossikov, and by Serebrovskaya and Shapiro.] In barley, Stadler (1929) found mutations to be much more readily produced in the cells of seedlings than in those of seeds. Aging spermatózoa greatly leads to a considerable increase in the frequency of mutations produced in them by X-rays, as Offermann has recently found (as yet unpublished), and cold accompanying X-raying markedly increases the X-ray mutation frequency, as Medvedev (1935) has recently proved, and as Papalashvilli (1935) had previously shown for the case of chromosome rearrangements. But that such influences affect the susceptibility of the gene to X-rays does not necessarily mean that the latter act indirectly ; it may only mean that the gene stability has somehow been altered.

Effects of Mutations on the Organism.

Let us turn now to consider the kind of effects on the organism which gene mutations produce. All mutation studies agree in showing that the different genes are individually very distinctive in regard to their possibilities of change, just as we should expect of chemicals so highly organized as present-day genes must be. Different genes and different mutated forms of the same gene differ very greatly both in their frequency of mutation and in the kinds of mutation which most usually occur in them (see especially the work of Stadler and that of Timofeeff-Ressovsky for exact studies of this principle). It is in fact the internal peculiarities of the individual gene rather than the nature of the impinging forces which define its mutational potentialities. But the kind of character effects in the organism which these mutations result in depends also upon the nature of the developmental reactions, through which the gene in question operates, and these reactions are of course conditioned by all the other genes as well. In this sense the genotype as a whole determines the nature of the mutational effects.

Although a given gene mutates preferentially in a certain way, to give a more or less extreme form of a given qualitative type, numerous cases are known of genes which can mutate in both of two opposite directions (as eosin eye in Drosophila to either white or red). And there are also many cases of genes being able to mutate in qualitatively different ways, and in different combinations of ways, so as to give rise to mutational forms that would never, either from their phænotypic appearance or from their mode of interaction with one another in compounds, have been thought of as derived from one another. The first and most notable case of this kind was that of the gene in Drosophila which mutates so as to give either truncated wings or a vortex on the thorax, or both, with or without a simultaneous lethal action. Genes may moreover undergo some internal change in configuration which alters their mutational potentialities, without altering their visible phænotypic effect (as in Baur's so-called " pre-mutations," as in the stable and " eversporting " allelomorphs of white pericarp in maize, or of miniature wing in *Drosophila virilis*, and as in Timofeeff-Ressovsky's too normal allelomorphs of white eye in Drosophila). And no doubt as step succeeds step in mutation and evolution, the further mutational possibilities of each gene continue to become greatly changed. From a technical genetic point of view, however, it would be very hard to demonstrate this later point directly, for it would be difficult to show that the latter mutations really lay at the locus of the

original gene, if they were very different from the earlier mutation in character and so did not show phænotypic signs of allelomorphism in the "compounds" produced by crossing them together.

When we consider not an individual gene but all the genes of the organism, the directions of character change which are open to an organism through mutation are of course vastly increased in number, but still mutation does not occur in all conceivable directions with equal readiness, and in some directions it does not occur at all, at least not by one step. Thus for example, while we find all degrees of eye colour ranging from white through yellow and red to purple and brown producible in Drosophila by a single mutational step from the normal red, nevertheless no greens or blues have yet been found. In cereals this principle has been illustrated on a grand scale, at least so far as mutations that have already been subjected to some selection are concerned in Vavilov's extensive studies in which he showed that when the whole series of varieties of related species are taken into consideration, they fall into great parallel groups—his principle of homologous variation. That is, the number of possible varieties, though great, is nevertheless limited, and very largely the same limitations are found in all nearly related species. There is no doubt that this principle holds even in the case of mutations that have not yet been subjected to any selection, although of course selection must be an added factor in producing such similarities. Such results are a reflection not only of the similarity of individual genes, which makes them subject to like mutations, but also of the similarity of the mechanism of development of the characters, which readily permits only certain kinds of changes in the end—results of the systems of interesting genes.

The conception of such a natural limitation of the paths of development is, however, very far from the conception of orthogenesis: according to which there is only one or at least one main direction in which genetic change can take place, this being the line which evolution is forced to follow. The latter idea, which fails to agree with the observed facts, would necessarily drive us back to mysticism in explaining how the direction of evolution in existing organisms could have been so elaborately adaptive as it obviously has been.

One general rule may be formulated concerning the preferential direction of mutation which apply to all organisms and nearly all characters. That is, that changes of the type which we may describe under the admittedly rather vague term of "degenerative" will be more frequent than other kinds. This does not at all imply that all mutations consist of losses of genes: in fact, we definitely know from the occurrence of reverse mutations, both spontaneously and as a result of X-ray treatment, that not all mutations are losses. And yet, as I have often pointed out, we should much more often expect mutations to work in the direction of apparent losses of *characters* (no matter in what sense we use the term "characters") than in the direction of gains or higher developments of characters. The reason for this is very similar to the reason for the second law of thermodynamics in physics, according to which, when physical changes take place, there is always a tendency for energy to become less concentrated in space, simply because, with many independent causes acting, there are so many directions in which a part of the energy might be sent, so as to result in its lesser concentration, than in its greater. Now in our genetic problem we are not dealing with concentration of energy in a spatial sense but in the sense of its qualitative direction, with respect to its working towards a given special end or "function": an end which meets the needs of the organism in that it furthers life. And there are, in general, many more ways in which changes can occur so as not to meet a given end so well, than ways which meet it better, and hence random changes will in general take the so-to-speak "downhill" direction.

Now the most general or all-inclusive character of an organism is life itself—that is, its "viability"—and since this depends on a highly complicated series of mechanisms that are the result of a long historical processe of selection, we may expect that lethal and other detrimental changes, leading to lesser viability, will be far more numerous than beneficial ones. But most individual characters of the organism are likewise the result of a high organization, though to a less degree than life as a whole, and so essentially the same principle will also apply to each of them, to a degree varying according to the degree of organization which is involved in their production. This leads to the proposition that the degree of development of these characters will more often

be weakened by mutation than strengthened. The same thesis applies not only to the final characters but to the various intermediary processes of physiology and development whereby they are produced and even to the first steps of their production. For the structure of the gene itself must, at the present stage of evolution, usually be very highly organized, and so it must usually be easier to change it to forms which will act more weakly in producing the product previously characteristic of it, than more strongly, and new products, likewise, would more readily be disorganizing rather than reorganizing in their effects, on the further reactions of development or of physiology. Mutations allowed to accumulate without selection should, therefore, result in those "losses" of function that attend disorganizational processes in general.

In correspondence with the above principles, we find that in fact most mutations do act in the direction of character losses. Moreover, when actual losses of genes are available for study and comparison with the effects of mutations in the genes, the latter are found, more often than not, to resemble these losses in their effects; that is, the class of mutations which I have termed "hypomorphic" (1932) is the largest.

These considerations in turn help to explain the observed fact that most of the mutations which the geneticist finds tend to be recessive in relation to the normal form. For if they commonly act somewhat like losses, then they should tend to be recessive if losses tend to be recessive; that is, if one dose of a gene commonly has an effect more nearly like that of two doses than of no dose. That the latter principle is true is demonstrated by a good deal of recent work. Wright had adduced reasons why this should be so on physiological grounds (since we should expect most curves relating the amount of a substance to its product to be convex rather than straight or concave, and since in a long chain of reactions there should seem to be much chance for an all-or-none effect to enter in somewhere). Whatever the validity of these reasons may be there is the additional reason that selection should tend to produce a type in which the effects of disturbances like those involved in alteration of gene dosage are minimized. Fisher thinks this selection works directly, since in any population individuals are regularly produced, in minute numbers, in which these very dosage changes have been produced by mutation. Both Plunkett and I, on the other hand, have been inclined rather to the view that other changes similiar *in effect* to those of dosage changes, would be more frequent, and that these would give more ground for a selective process having this same end result. At any rate, all the factors mentioned, in so far as they are operative at all, lead towards the dominance of the normal gene over its mutant. And on any selection view this would hold true the more strongly in the case of changes which were larger, more detrimental and more frequent; this means especially those having a character like losses.

Genetics has arisen historically in connection with the morphological rather than with the physiological biological sciences, and so geneticists have not usually realized sufficiently that the readily visible morphological aspects of vital phenomena constitute a relatively small fraction of life as a whole. From this point of view, we see that readily visible morphological mutations would probably be but a small fraction of all the mutations occurring. It was shown some time ago in Drosophila that lethals were from five to ten times more frequent than the readily visible class of mutations and, as Kerkis, Timofeeff-Ressovsky and several others have recently shown independently in Drosophila, demonstrable mutations that are neither fully lethal nor productive of visible effects are two to three times as frequent as fully lethal mutations. It is not unlikely that there is a still larger class of mutations that have effects too slight or too hidden to have yet been demonstrable. Moreover, in the case of the great majority of morphological characters, mutations occurring within the range of detectibility are apt to be less frequent than the slighter ones, for the organism has accumulated by many small evolutionary steps, involving changes in many genes affecting the given character.

In general there will be a considerable correlation between the degree of a change and the amount to which it is detrimental. That is, larger changes will, on the average, be more harmful, while conversely there will be more chance for smaller changes to be constructive or reorganizing rather than disorganizing. For these reasons, as was pointed out long ago (1921 *et seq.*) by Sturtevant,

Bridges, Baur and myself in separate publications, evolution may be expected to proceed much more through selection of many minute so-to-say "quantitative" mutations than of conspicuous mutations of the sort with which geneticists have found it most convenient to deal. The results of species crosses have long been known to confirm this conception. Now an examination of the discussion above given concerning dominance will show that the principles there involved tend to apply more strongly to the more conspicuous and to the more degenerative mutations than to the minute ones, or to those of less detrimental action. Thus smallness of effect, less harmful character, and lesser recessiveness of mutations all tend to go together.

East (1935) has very recently called our attention again to this preponderant importance of the small mutations as material for evolution and to their lesser dominance. But the workers with Drosophila and Antirrhinum, those dealing with the mathematics of selection, and various others, have already stood on this ground for a long time, and in accordance with this they have maintained that the mutation theory carries us back to the essentials of Darwinism. They have pointed out that it in fact provides a far firmer foundation for the idea of evolution through selection of small differences than Darwin ever had. The first reason for this is because it eliminates his main difficulty, that of the "swamping out" of small variations in crosses, inasmuch as the mutated genes are inherited in a Mendelian manner. The second reason is that the results of investigations concerning mutations provide direct evidence of his thesis of the accidental, non-purposive, non-directive nature of the hereditary variations of evolution.

Changes in Gene Arrangement.

Among the phenomena classed by De Vries as "mutations" were cases which we knew to depend upon changes in the number of sets of chromosomes (polyploidy), in the number of individual chromosomes (heteroploidy or alloploidy), and other changes which we now know to have been in the arrangement and number of chromosome parts, that is, of whole blocks of genes. He tended to think that all these were merely expressions of more deep-seated genetic alterations, but we now know them to be of an "autonomous" nature. As for the rôle of such changes in evolutionary differentiation, it has been shown that polyploidy and to a lesser extent heteroploidy sometimes plays a decisive part in species formation, especially in the case of cultivated plants. It is easy to reckon, however, that of all the hundreds or thousands of species-transitions and of the millions of individual mutational steps, that have occurred and become established in the long line of ancestry of any now existing species of higher plant or animal, the number of occurrences of polyploidy or heteroploidy must have been exceedingly small, even though it is just these effects which remain to this day the most conspicuous cytologically.

Rearrangements of chromosome *parts*, however, are of far more varied kinds, for there are thousands of "interloci" at which a break in a chromosome may occur, and other thousands at which a second break, determining the point of reattachment of the genes detached by the first break, may simultaneously occur. For this reason, and because of the fact that these rearrangements may occasion little or no change in gene number (changes in the number of many genes in relation to other genes being too upsetting to the normal balance of developmental reactions), these rearrangements of chromosome parts have far more chance of becoming established in evolution than changes in the number of whole chromosomes or sets of chromosomes.

Since the finding that rearrangements of chromosome parts are readily producible by X-rays, their origination has been subjected to considerable investigation. It has been found that in the vast majority of cases these rearrangements of genes involve an exchange of the connections of the chromosome threads (gene chains) between two or more points at which they underwent breakage: the process, in other words, is somewhat like a kind of "illegitimate crossingover," one occurring between non-homologous instead of homologous regions of chromosomes (Muller, 1932). Very few cases are known which can be interpreted on the basis of a chromosome having simply become broken at one point into two pieces (with resultant loss of the fibreless piece), or on the basis of a simple breakage of one chromosome, followed by the attachment of one of the pieces to the end or side of another unbroken chromosome or chromosome region. Most earlier supposed

cases of this kind have been found to involve misinterpretations. And even those few cases still remaining as possible exceptions to our rule are open to the suspicion that what really happened was the exchange of connections at two points of breakage, one of which, however, happened to lie too near the end of the chromosome to be detectible as such. (A discussion of this matter has been given in a paper by Prokofyeva, Belgovsky and Muller, in press.) On the basis of these findings, we may conclude that both the free end of the chromosome and the point of spindle-fibre attachment are comparatively permanent structures which remain as such even through numerous rearrangements of chromosome parts.

According to the morphology of the exchange, mutual translocations, inversions, deletions, insertions, duplications, etc., are produced. Later, when recombination of chromosomes occurs at cell division, especially at maturation, there is an opportunity for certain lines of cells, and the individuals developing from them, to inherit extra blocks of genes, or to lack blocks of genes. Homozygosis for a missing section of a chromosome causes inviability (except in rare cases where the section is exceedingly minute), but an individual homozygous for an extra section may much more often be viable, provided the section is not large (a result which is of course dependent also upon the individual peculiarities of the genes included). In this way, a method is afforded whereby the number of genes can be increased in the course of evolution, as the author has pointed out in connection with cases of increase of genes arising in laboratory cultures. In the meantime, Bridges' detailed studies of the structure of the salivary gland chromosomes of *Drosophila melanogaster* (following Painter's already classic method) demonstrated the existence of two comparatively large sections which had evidently become duplicated in this way in the past evolution of our present-day normal Drosophila. Similar studies in our laboratory by Offermann and by Kossikov have demonstrated two additional cases of this kind. This shows, then, the way in which the genetic basis of organisms can increase in quantity in the course of evolution, becoming more compound. It is to be expected that later, through differential mutations in this larger collections of genes, an increase in complexity of the genetic basis, and, through this, of the phænotypic superstructure, can follow.

Translocations and inversions that involve no change in gene number can also be of evolutionary significance. This may happen in two ways. Firstly, as I pointed out in 1928, mechanisms of partial genetic isolation between the new form and the one from which it sprang are thereby provided. Secondly, as has been proved by recent work of a considerable number of investigators, the rearrangements may be of direct selective significance through the fact that such changes influence the functioning of genes near the point of rearrangement—a phenomenon known as the "position effect". Rearrangements, however, provide only one means of genetic isolation, and a partial one at that, and there is no doubt that species differentiation may take place without it, especially, if there is geographical isolation. And as for the position effect, while that is very real and must be taken into account, it can easily be shown that changes due to this cause must be far more limited in their variety and their potentialities than those due to mutation within the gene itself, and that they can for this reason be expected to supply only a small fraction of the material of evolution (see also the statement of this matter by Offermann, 1935).

In view of the latter considerations, it will be very important to determine what proportion of the mutations produced by X-rays are really nothing but gene rearrangements accompanied by position effects, rather than mutations within the gene. This question has become acute lately, since our finding (Muller, Prokofyeva and Raffel, 1935) that there is a class of gene rearrangements which are very minute, and the position effects of which (or, in the case of minute deletions, the effects of the losses of whose genes) may easily be mistaken for the effects of mutations within the genes. It has even been questioned whether perhaps all apparent "gene mutations" produced by X-rays really belong in this category. One method by which we are now trying to throw light upon this question is by a study of the frequency of cases known to be minute rearrangements, in relation to the dosage of X-rays used. As stated in a preceding section, the frequency of apparent "gene mutations" is

in direct proportion to the dosage of irradiation. On the other hand, the frequency of gross rearrangements is not proportional to the dosage, but varies more strongly than the latter, though not as strongly as the square of the dosage. This has been shown for deletions by myself and Koerner, for translocations by Belgovsky, and for inversions by Berg and Borisoff.* If now the minute rearrangements are found to follow the same rule as the large ones, this will constitute evidence that the apparent gene mutations really do belong in a different category from rearrangements. But if the minute rearrangements, like the apparent mutations, follow the simple proportionality rule, we shall be confronted with a much harder problem in deciding to what extent X-ray mutations in general are intragenic. The importance of this question is heightened in consideration of the fact that the X-ray mutations so far found strongly resemble spontaneous mutations, and that we have been able to find, among the results from X-rays, every type of mutation that we have searched for that was previously known to have occurred spontaneously.

Gene Mutations as the Primary Steps of Evolution.

But whatever the answer may be to the questions above raised, there can to-day remain no doubt that changes within the genes, that is, sudden mutations—since geneticists in all their searchings have found no evidence of gradual gene changes,—form the main basis of species differentiation and hence of all biological evolution. This has been abundantly proved by the species crossings which many botanists and some zoologists have performed in which, for one reason or another, chromosome segregation was prevented. There are many ways of preventing chromosome segregation. Among these are amphidiploidy, non-pairing of chromosomes followed by their division at both maturation divisions as in Federley's crosses of butterflies, a sexual reproduction of different kinds, linked chromosomes as in Oenothera, and chromosome combinations such that only those of the original species and the hybrids are viable. Now whenever any of these conditions has rendered it difficult or impossible for chromosome recombination, that is, Mendelian recombination of genes, to occur, there has been little or no spread of variation on breeding the hybrid. But where these processes of gene recombination were not prevented, then the hybrid, on being bred, has produced a second generation which shows a spread of variation, with the eventual possibility of return completely to the type of either parent species. These series of facts, taken together, prove that all the differences between the species (aside from the comparatively rare differences that seem to be dependent upon plastids) depend upon chromosomal Mendelian genes, and therefore, like all Mendelian differences whose origin is known, arise by some process of gene mutation.

There is also evidence which shows that gene mutations that have become common or well-established in a given group of organisms, and which are accordingly in harmony with one another, do not always work out advantageously when in combination with genes that have become established in a related group. In this way, inviability or sterility of the first or later hybrid generations may result. Thus, even without chromosome rearrangements, we have in gene mutation itself a mechanism for species splitting, as well as for the whole long line of evolutionary progression.

The usual mutation is, as we have seen, detrimental. And so it can only be by virtue of selection that the rare advantageous one, unlike the majority of mutations, takes part in evolution, and that progressive adaptation ensues. Selection thus remains the guiding factor which has been responsible for adaptation, and without which no elaborate adaptation could have occurred. This brings us back again to the principles of Darwinism, but a Darwinism far more firmly founded than that of the past century.

* The reason for the gross rearrangements varying more strongly than the dosage may lie in their being, to some extent at least, the product of two more or less independent events, namely, the breakages at the two or more points which undergo exchange with one another. This, if true, would mean that the exchange is not (always) caused by a crossing of the threads, as Serebrovsky and Dubinin (1930) had postulated, but that, as was maintained by Stadler (1932), the threads may break first and then afterwards find one another and become attached again at the points of breakage. Further evidence favouring this latter interpretation is to be found in the fact that, on the view of *a priori* touching of the threads, it would often have to be assumed that more than two threads crossed at exactly the same point, since it is now known that more than two threads often undergo a mutual exchange. (See my provisional arguments on this point in the *Proc. of the Genetics Congress*, 1932, and in paper on the scute—19 insertion, 1935.)

CYTOLOGY OF SEX.

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(*Received 29th August 1936.*)

THE introductory studies by Henking (1891) and by McClung (1902) on sex-determining chromosomes in insects, and particularly the classical investigations by Wilson on sex chromosomes in the two bugs Protenor and Lygæus (1905), threw the first light on the cellular mechanism that leads to the appearance of the two kinds of individuals, males and females.

Wilson's demonstration of the fact that the nuclei of the somatic cells in the male Protenor embody 12 ordinary chromosomes (autosomes) and 1 large so-called *X*-chromosome, while in the female there are 12 autosomes and 2 *X*-chromosomes, was of epochal significance. The principle of sex-determination was thus established. In the formation of sex cells the chromosomes were distributed through the so-called reduction division by which the chromosome sets were halved, so that the spermatozoa of the male turned out to be of two kinds with respectively $6 + X$ chromosomes and 6, while the eggs of the female all had $6 + X$ chromosomes. On fertilization of the eggs the result would then again be two kinds of individuals, namely, some with $12 + X$ (males), and others with $12 + 2\,X$ (females). The male Protenor, giving two kinds of sex cells, is called heterogametic, the female homogametic.

Wilson found a variation of the same principle in Lygæus, where the male possesses, besides $12 + X$, an additional little so-called *Y*-chromosome, which appears as the partner of the *X*-chromosome, and is absent in the female. As in the case of Protenor, the female Lygæus has $12 + 2\,X$. The difference between the two organisms is meraly this: in Lygæus the sex cell of the heterogametic male have respectively $6 + X$ and $6 + Y$, while in Protenor they have respectively $6 + X$ and 6. In Lygæus thus the full set of chromosomes in the male is $12 + X + Y$, in the female $12 + 2\,X$.

Apart from the fact that it is not practicable in all species to distinguish between *X* and *Y*, nor to differentiate these chromosomes from autosomes, and leaving out the condition in which one of the sex chromosomes may be represented by two or more little chromosomes that keep together in a group, the principle here outlined applies to all organisms in which fertilization of the egg leads to the production of male and female individuals.

As far as man is concerned it is not yet known with certainty whether the sex differentiation be of the Protenor type or of the Lygæus type. That the man is heterogametic and the woman homogametic has been established with certainty, but only through genetic investigation. Several instances of sex-linked inheritance, in particular of genes linked to the *X*-chromosome, show that women have 2 *X*'s and men only 1 *X*. The cytological studies carried out through the last 15 years by Winiwarter and Oguma, by Painter, by Evans and Svezy and by Minouchi and Ohta strikingly illustrate the difficulty of identifying the sex chromosomes in an organism which possesses as many chromosomes as man (about 24 pairs). The variance among the observations involves not only whether or not there be a *Y*-chromosome in man, but also whether the *X*-chromosomes belongs among the largest chromosomes or among the middle-sized.

In plants the occurrence of sex chromosomes was not established till 1917 when Allen demonstrated that the moss *Sphærocarpus* belongs to the *Lygæus* type. In dicœcious flowering plants sex chromosomes were observed for the first time in 1923 (Santos, Kihara and Ono, Blackburn, Winge).

From the findings reported so far, it appears as if the male is most often heterogametic, in the animal kingdom as well as among the plants. The reverse is the case, however, in birds, butterflies, certain fishes, the caddis-fly (Limnophilus) and, in the plant kingdom, in strawberry (Fragaria). Undoubtedly, this list will gradually be increased with other organisms.

It is particularly in studies on Drosophila that attempts have been made to establish where the sex-determining genes are located. The appearance of individuals with deviating chromosome sets has led to the view that the *X*-chromosomes here pull in the feminine direction, while the *Y*-chromosome of the male contains no sex-determining genes. In the normal banana-fly the chromosome number is $6 + 2\,X$ in the female, and $6 + X + Y$ in the male. Individuals which

through faulty distribution of the sex chromosomes in the reduction division of the egg have got $6 + X$ turn out to be males, notwithstanding the absence of a Y-chromosome; and individuals with $6 + 2X + Y$ turn out as females, in spite of the presence of the Y-chromosome. In Drosophila, then, the sex-determination would be decided by the circumstance that the masculine element in the autosomes is stronger than the feminine element in one X-chromosome, but weaker than the feminine element in two X-chromosomes. Also the sex in individuals with triploid sets of autosomes and varying sets of sex chromosomes has corroborated with this view.

The general theory of sex-determination as worked out and formulated by Goldschmidt, Hartmann and, particularly, Bridges says that all individuals contain both masculine and feminine elements. If the masculine are predominant, the result is males; if there is an overweight of feminine elements the result is females. The comprehensive experiments carried out by Goldschmidt with the butterfly Lymantria have greatly helped to elucidate the competition between the two sorts of genes pulling in opposite directions. By crossing different races of Lymantria, deviating mutually by differences in the strength of the sex-determining genes, Goldschmidt succeeded in producing sexually abnormal types, intersexes, the appearance of which is attributed to an abnormal balance between genes that are pulling in the male direction and genes pulling in the female direction.

Opinions differ considerably as to the location of the sex-determining genes and their numbers. Morgan maintains the general view as outlined above: that in principle the balance between feminine elements in the X-chromosomes and masculine elements in the autosomes is decisive in sex-determination—at any rate, in the banana-fly. Witschi thinks this theory will apply to all organisms. In the course of time, Goldschmidt has varied his view as to the location of the sex genes, and he assumes, among other things, that the nature of the cytoplasm plays a part in the sex-determination and is able, besides, to strengthen or weaken the effect of the sex genes located in the chromosomes. Kosswig thinks that in the fishes he is working with, the masculine element is to be assigned to the Y-chromosome and the feminine element to the autosomes; he thinks the X-chromosomes in these species are empty.

According to the general theory of sex-determination advanced by Bridges (1921, 1932), all chromosomes contain sex genes, some pulling in the male direction, others in the female. Dobzhansky and Schultz (1934) have worked with the banana-fly *Drosophila melanogaster*, in which there may occasionally occur translocation and duplication of small chromosome segments which are recognisable as definite portions of the X-chromosome. These authors think that they have been able to demonstrate that several genes, pulling in the female direction are located in different parts of the X-chromosome. But Goldschmidt (1935) disputes the validity of the interpretation of these experimental findings.

Experiments with the tropical fish *Lebistes reticulatus* (1922–34) have led the present writer to the conclusion that the principle of Bridges' theory is correct, and that the other theories concerning sex-determination have to be replaced by a theory based on this fundamental view.

In Lebistes the female has XX-chromosomes, the male XY. A considerable number of genes for colour patterns are located in X and in Y, and some of them are able to cross over from X to Y and *vice versa*, while others are firmly connected with the Y-chromosome; owing to these circumstances it has been possible to establish the occurrence of individuals in which the sex is in contrast to their sex-chromosome equipment, and thus to elucidate the cause of disagreement between sex-chromosome equipment and sex.

Some XX males of irregular occurrence were found on crossing with normal XX females to give all daughters. In order to obtain again some XX males, some of these daughters were backcrossed with their XX father. Still, this backcross gave anew all daughters; but by backcrossing some of the latter with XX males the result was many daughters and one new XX male. When this male was mated with its mother, the offspring showed about 50% of either sex. Evidently, the explanation is: that XX males contain such a large amount of autosomal genes which pull in male direction that they overmatch the feminine X genes in strength. It may be taken for granted that a pair of autosomes have taken over the rôle of a pair of sex chromosomes, for

when the offspring shows about 50% of either sex there must be a functional mechanism of sex-determination. The *X*-chromosomes are put out of the game as sex-determinants, for males as well as females have *XX*, and, as was to be expected, the genes located in the *X*-chromosomes are transmitted in an ordinary Mendelian, not sex-linked manner. It should be mentioned, however, that the sex-determination in the *XX* race is less sure than in normal Lebistes. It is quite true that the animals develop into pure males and females, but towards the end of the summer and, especially, in the winter there are born far more females than males, so that it is reasonable to assume that external conditions are able to make the sex-determination tip in the "wrong" direction. What external conditions are here involved is yet an open question. By means of a theoretical example, I shall demonstrate what I think may be the explanation of this experiment.

In normal Lebistes we assume there is a female (negative) tendency in the *X*-chromosomes and a male (positive) tendency in the *Y*-chromosome, while the other chromosomes, of which we here consider only 6 pairs (A—F), contain a relatively large number of sex genes, most of which have only a relatively weak effect, some in a female (negative) direction, others in a male (positive) direction.

Individuals with a positive total sum of genes are males; those with a negative sum are females. Autosomes with positive, masculine effect are here designated by small letters; autosomes with negative, feminine effect are designated by capitals.

A Lebistes race, picked out at random, has, for example, the formulæ:

X	*X*	*A*	*a*	*B*	*B*	*c*	*c*	*D*	*D*	*E*	*e*	*F*	*f*
−	−	−	+	−	−	+	+	−	−	−	+	−	+
12	12	20	3	6	6	4	4	2	2	3	2	1	1

Total: + 14 − 64 = − 50 (♀)

and

X	*Y*	*A*	*a*	*B*	*b*	*c*	*c*	*D*	*d*	*e*	*e*	*F*	*F*
−	+	−	+	−	+	+	+	−	+	+	+	−	−
12	70	20	3	6	3	4	4	2	3	2	2	1	1

Total: + 91 − 42 = + 49 (♂)

To any geneticist it is obvious that—provided the theory holds good—it will be possible by suitable selection among the descendants of these two animals to obtain *XX* females and *XX* males of the following formulas:

X	*X*	*a*	*a*	*b*	*b*	*c*	*c*	*d*	*d*	*e*	*e*	*f*	*f*
−	−	+	+	+	+	+	+	+	+	+	+	+	+
12	12	3	3	3	3	4	4	3	3	2	2	1	1

Total: + 32 − 24 = + 8 (♂)

and

X	*X*	*A*	*a*	*b*	*b*	*c*	*c*	*d*	*d*	*e*	*e*	*f*	*f*
−	−	−	+	+	+	+	+	+	+	+	+	+	+
12	12	20	3	3	3	4	4	3	3	2	2	1	1

Total: + 29 − 44 = − 15 (♀)

Crossing these two animals will necessarily give 50% of either sex, and now the *X*-chromosomes are no longer the sex-determinants, but this function is accomplished by one pair of the autosomes, A—*a*.

At the same time the sex difference is less pronounced, as it has fallen off from 9 to 23, and hence there is now the possibility that extenal conditions—in keeping with the experimental findings—may bring about a shift in the determination of the sex.

It will be noticed, moreover, that now the female is heterogametic, while the male is homogametic. In brief: Sex chromosomes have changed to ordinary autosomes, a pair of autosomes to sex chromosomes, male heterogamy to female.

XY females were also seen occasionally in Lebistes; and it was predicted—and then verified—that these deviating females would give three times as many males as females in the offspring when they were crossed with normal *XY* males. In the first control experiment the result was 81 males and 28 females, and the colour of the males showed that 34 possessed the paternal *Y*, 25 the maternal *Y*, and 22 had a *Y* from both the father and the mother. The last-mentioned individuals thus are males with two *Y*-chromosomes and no *X*. These *YY* males are perfectly viable and capable of fertilization. When mated with normal females they naturally give only male offspring, for all the offspring must have a *Y*-chromosome.

By this we arrive at the other possibility for changing male heterogamy to female. We now have males with *YY* and females with *XY*. By all the signs it will be possible to stabilize this state of conditions, even though it has not been accomplished yet. By repeated backcrossing of *YY* males with *XY* females it should be possible to introduce so many autosomal feminine genes in the offspring that the balance in future will be adjusted so that *YY* individuals are males and *XY* animals females.

Recently Aida, in Japan, has obtained corresponding results with another fish species, Aplocheilus : and it may be added that Goldschmidt's results with Lymantria, as well as other similar findings of this kind may be explained readily in the same manner.

From the studies mentioned above, I think, that there can hardly be any doubt that sex genes are present in all chromosomes, and that there is always the possibility that sex chromosomes may change to ordinary autosomes, and *vice versa*.

If the autosomes contain a large amount of relatively weak sex genes which counterbalance each other fairly well, there is the possibility that crossing-over between these genes may result in autosomes with strong masculine or feminine effect. Such autosomes can take part in sex-determination, and they may eventually take over the rôle of sex chromosomes.

It must be added that many observations suggest that *X*-chromosomes and *Y*-chromosomes in a given species have greater or lesser parts in common. This is shown by the fact that *X* and *Y* are paired during the reduction division, as well as by the circumstance that crossing-over between them may take place (in Lebistes and others). Possibly they differ sometimes only for a short distance.

In this brief review I shall merely mention the greater deviation in cytological conditions found in Pseudococcus (Schrader, 1921, 1923) and other organisms, in which the chromosomes originating from the father or from the mother appear to keep together as a unit (attachment of non-homologous chromosomes), hence all the chromosomes so to speak, are sex chromosomes.

The same applies to the conditions in the dipteron Sciara (Metz, 1934 and before), in which the fertilization of the egg is followed by an elimination of chromosomes that influence sex-determination.

Nor shall I here enter into the special conditions found in ants, bees, wasps and others in which the males come from non-fertilized eggs and thus are haploid, while the females come from fertilized eggs and thus are diploid. One of the most difficult problems at present is, to find an explanation for why diploidy *per se* brings about female sexuality, haploidy male sexuality. This often seems to be the case in organisms with haploid males and is hard to reconcile with the view that sex-determination is decided by the balance between the genes pulling in a female direction and the genes pulling in a male direction. *A priori* it is not to be expected that a doubling of the chromosome sets would bring about a new balance. On the basis of experiments with Habrobrachon Whiting (1935) has set forth the hypothesis that there are two kinds of haploid males, some with *X* and some with *Y*, and that diploid *XY* individuals turn out as females, on account of a complementary interaction between *X* and *Y*. But this hypothesis, I think, calls for additional assumptions of such scope that it is not likely to gain foothold.

CYTOGENETICS OF SPECIES HYBRIDS.

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(*Received 28th September 1936.*)

STUDIES of species hybrids were undertaken many years ago. *Linné* was the first biologist to record natural interspecific hybrids. He even tried to obtain artificial hybrids. However, the heredity of species hybrids has scarcely been investigated until recently. Because of the difficulties of genic analysis, it appeared to be lawless and fortuitous. The rapid progress of cytogenetics in the last two decades has proved that not only chromosome numbers in pure species, but chromosome behaviour at maturation divisions in hybrids give valuable informations toward the solution of puzzling problems in genetics.

Cytogenetical work has largely dealt with groups of plants showing polyploidy, for instance *Avena*, *Triticum*, *Nicotiana*, *Rosa*, *Brassica* and *Crepis*. The author has studied wheat and its relative genera (*Aegilops*, *Secale* and *Haynaldia*), and will attempt to give a short description of the present status of this problem from his own experience.

Interspecific hybrids exhibit a range of sterility, and the degree of sterility is in most cases correlated with the affinity of the parents. Complete sterility appears to be rare. Even highly sterile plants give offspring if extensive material is used. In some cases complete sterility is caused by the abortion of androœcium and gynœcium.

In plants partial fertility is readily demonstrable by an examination of pollen grains. Owing to the non-dehiscence of the anthers, which contain a few good pollen grains, the sterility is attributed to the male. Nevertheless the percentage of functional gametes seems to be the same in both sexes. But the percentage itself varies greatly with different external conditions. In a hybrid (*Aegilops caudata* × *Ae. speltoides*) there was a marked difference in the percentages of good pollen grains obtained from plants cultivated in pots and from those in the field.

In a hybrid within a species, the maturation divisions proceed quite normally and accordingly the number of chromosomes in the offspring is the same as that of the parents. An interspecific hybrid which has normal chromosome behaviour and normal fertility can be treated as an intraspecific hybrid in spite of the taxonomical position of the parents.

For sake of convenience, we can divide species hybrids into two groups:

(1) Hybrids with at least two homologous genoms.[1]

(2) Hybrids without homologous genoms.

This classification is based on the mode of conjugation of chromosomes from different parents. The hybrids of the first category must have at least three genoms (sets of chromosomes), because if there are only two homologous genoms, and accordingly no univalent is found, the hybrid can be treated as an intraspecific hybrid. The hybrids of the second category may be diploid or polyploid.

Behaviour of Hybrids belonging to the First Category.

Extensive investigations were made of this type of hybrids in the pentaploid wheat hybrids (*Triticum duum* × *vulgare*). In the first maturation division of this hybrid, there are 14 bivalents and 7 univalents ($14_{II} + 7_{I}$). The 14 bivalents behave normally throughout the meiosis. But the univalents split equationally in the first division and are distributed apparently at random in the second. The gametic chromosome number may be represented by $14 + i$, where i has values ranging from 0 to 7 with frequencies corresponding to $(a + b)^7$. The value of a and b is 0·5, if the distribution of univalents is at random and there is no loss of univalents. There are some irregularities such as lagging of the daughter halves of the univalents, which cause elimination and fragmentation of the chromosomes. The values of a and b are therefore not equal. By backcrossing experiments a is calculated to be approximately 0·7, when strong elimination, and 0·6, when moderate loss of univalents takes place. The actual frequency of the gametes is somewhat different from that expected from the expansion of $(a + b)^7$. The frequency polygon is very flat.

Since the same series of gametes occurs in both pollen grains and ovules, chromosome

[1] Genom = Haploid set of chromosomes.—*Ed.*

numbers ranging from 28 to 42 are expected in F_2. We have really found the complete F_2 zygotic series. The distribution of the individuals with different chromosome numbers looks like a frequency polygon with a slight indication of bimodality. But if we compare the actual frequency with the one calculated from $(a + b)^7 \times (a + b)^7$, there is a marked difference (Fig. 1). The former is

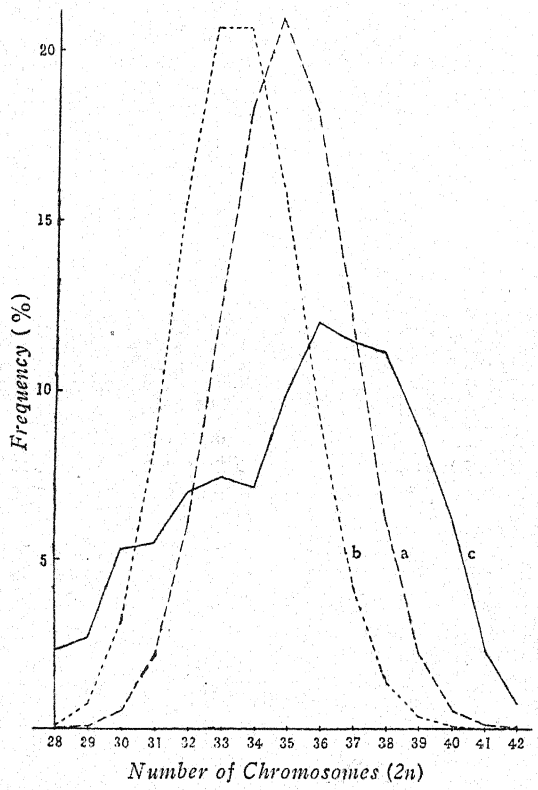

Fig. 1.

Theoretical and observed frequency of 28–42, chromosomes in F_2.

a.–Theoretical frequency calculated from $100 \times (0{\cdot}5 + 0{\cdot}5)^7$.
b.–The same from $100 \times (0{\cdot}6 + 0{\cdot}4)^7$
c.–Observed frequency. (After Matsumura.)

too flat. Three reasons can be given for this difference, *i.e.* :

(1) Selection of male gametes in favour of those with chromosome numbers just as many as the parents or nearly so.
(2) The elimination of zygotes with non-viable chromosome combinations (Table I).
(3) Selective fertilization of gametes.

A detailed explanation of this subject is beyond the scope of this review.

TABLE I

The Possible Chromosome Combinations in F_2 Progenies of Pentaploid Wheat Hybrids (after Kihara).

Chromosome Numbers of F_1 and F_2	Viable Combinations I	Non-viable Combinations II	III	IV
28	↑ 14II+			
29	14II+1I			
30	14II+2I	15II+		
31	14II+3I	15II+1I		
32	14II+4I	15II+2I	16II	
33	14II+5I	15II+3I	16II+1I	
34	14II+6I	15II+4I	16II+2I	17II
35→ 35	14II+7I	15II+5I	16II+3I	17II+1I
36	15II+6I	16II+4I	17II+2I	18II
37	16II+5I	17II+3I	18II+1I	
38	17II+4I	18II+2I	19II+	
39	18II+3I	19II+1I		
40	19II+2I	20II		
41	20II+1I			
42	↓ 21II+			

The viable combinations are grouped into two classes : one ranging in chromosome numbers from 28 to 34, called the diminishing group, because in subsequent generations the chromosome number gradually reverts to 28 ; the other with chromosome numbers 36–42 called increasing group, becuase their progeny returns to the 42-chromosome condition of the other parent. The reversion of chromosome number to the parental condition is very rapid in the diminishing group and rather slow in the increasing group. The reason may be sought in the loss of univalents.

The fertility of F_2 plants with different chromosome numbers is very interesting (Fig. 2). It varies hand in hand with the

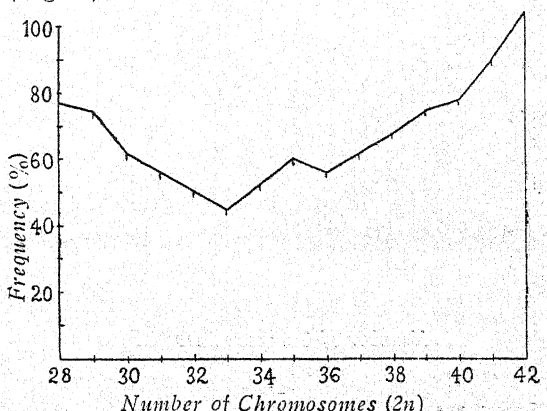

Fig. 2.

Curve showing fertility of plants with different chromosome numbers in wheat. (After Matsumura.)

chromosome number. Generally, the fertility of plants with the parental chromosome numbers is perfect or nearly so. The whole process leads to the final reversion of the descendants to the parental chromosome condition.

The genic analysis of the pentaploid hybrids revealed that the genes located on the bivalents are transmitted quite independently of the fluctuation of the chromosome numbers, while those located on the univalents are associated with the characters of the *vulgare*-wheat.

Occasionally, stable lines with non-viable chromosome conditions were found. They were highly sterile and characterized by some morphological characters such as dwarfness. In some cases the chromosome condition was restored from 20_{II} to 21_{II}. The increase of one pair was the result of (doubling of one) chromosome of the A or B genoms probably by aberration. Parallel phenomena were found later in different interspecific hybrids, *e.g.*, triploid Avena- and Allium-hybrids (also many unpublished data). They also gave in subsequent generations diploid or tetraploid offsprings, which were fertile and constant in chromosome number. It is quite possible that this rule governs any hybrid of the first category.

It is interesting to note that the fertility of gametes with intermediate chromosome numbers rises with the number of complete genoms. Accordingly, the fertility of triploids is generally lower than that of pentaploids (Fig. 3).

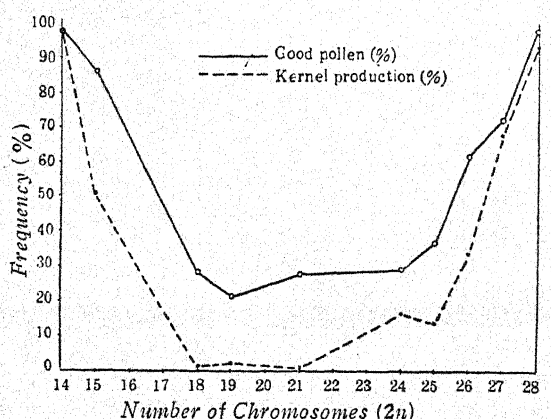

Fig. 3.

Percentages of good pollen grains and fertility in plants with different chromosome number in oats.

(After Nishiyama.)

Behaviour of Hybrids with Non-Homologous Genoms.

The behaviour of these hybrids is quite different from that of those mentioned above. In most cases only the gametes with unreduced chromosome number are functional. They give therefore offspring with a chromosome number twice that of F_1 which are constant both in chromosome number and morphology.

Here again we see that the balance of chromosomes is obtained only when the genoms are complete. But if one of the genoms, for example A in a diploid hybrid AB, be complete, the gamete should be normal. Theoretically, the random segregation of bivalents and univalents in a diploid hybrid with genom type AB gives in rare instances, the parental chromosome combination, A or B. The percentage of gametes with one complete genom (G) can roughly be calculated by the formula,

$$G = \frac{1}{2^{p+q}} \times 100,$$

where p = number of bivalents and q = number of univalents at the mode. The theoretical expectation is not yet fully confirmed. In one case, *Triticum ægilopoides* × *Aegilops squarrosa*, it proved to be true (Kihara and Lilienfeld, 1935).

This hybrid has in meiosis $(0-6)_{II} + (14-2)_I$ and the mode is $2_{II} + 10_I$. (Table II). The calculation is made as follows[2]:

$$\frac{1}{2^{10+2}} \times 100 = \frac{1}{4096} \times 100 = 0{\cdot}024\%.$$

Table II.

Frequency of Cells with 0 – 6 Bivalents in PMC of Triticum ægilopoides × Ægilops squarrosa.

Bivalents ..	0	1	2	3	4	5	6	Total
Frequency ..	12	18	25	22	12	9	2	100

[2] For an exact calculation, the following formula should be used :—

$$G = 100 \times \frac{1}{N}\left(\Sigma \frac{x}{(2)^{p+q}}\right),$$

where x = frequency of PMC's with different chromosome number and $N = \Sigma x$ = total number of PMC's.

$$\therefore G = \left(\frac{12}{2^{14}} + \frac{18}{2^{13}} + \frac{25}{2^{12}} + \frac{22}{2^{11}} + \frac{12}{2^{10}} + \frac{9}{2^{9}} + \frac{2}{2^{8}}\right)$$

$$= \frac{12+18\times2+25\times4+22\times8+12\times16+9\times32+2\times64}{2^{14}}$$

$$= \frac{932}{16384} = 0\ 057\%$$

One F_2 individual with a full double *ægilopoides* genom (AA) was obtained from open pollination of 2284 florets (0·04 %). Therefore it seems to be correct to say that the difference between the expectation and the result obtained is not significant. The verification of this hypothesis from extensive backcrossing experiments is not yet complete. However, the following conditions must be considered :

(1) Technical difficulties in artificial pollinations. The success in getting seeds (%) varies to a great extent.

(2) The calculation is based on the chromosome conjugation in PMC of F_1. In some hybrids, some difference can be found between PMC and EMC.

(3) Regression may disturb the random segregation.

(4) Crossing-over between partially homologous chromosomes can give higher sterility than we expect.

In this connection, a monogenomic haploid plant is a suitable material for this purpose. The results obtained from a haploid einkorn (*T. monococcum*) backcrossed to normal diploid *monococcum* show that the expectation is fulfiled.

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THE GENETICS AND CYTOLOGY OF CITRUS.

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(Received 5th March 1937.)

Introduction.

THE following discussion relates mainly to the genus *Citrus* in the modern sense (*Eucitrus*), with occasional references to two genera, *Poncirus* (the trifoliate orange) and *Fortunella* (the kumquats), which are so closely related to *Citrus* that they can be intergrafted and intercrossed. All "citrus" forms of major importance in fruit production belong to the genus *Citrus*; certain kumquats and intergeneric hybrids of *Citrus* are of minor significance in fruit production, and *Poncirus trifoliata* Raf. is extensively used as a stock for *Citrus* and *Fortunella*.

Polyembryony.

Polyembryony occurs in most but not all forms of *Citrus*, and also in *Fortunella* and *Poncirus*. It is brought about in two ways: (1) normally and often abundantly by an asexual process, nucellar embryony; (2) occasionally by fission of the generative (sexually produced) embryo.

Embryonic Fission.—Rarely a *Citrus* seed produces two generative seedlings, and in such cases the two seedlings appear genetically identical. In view of the great diversity of the generative seedlings from different seeds of any polyembryonic *Citrus* form, it is extremely probable that each pair of such "identical twins" is derived from one fecundated egg.

Nucellar Embryony.—Extra embryos of the usual kind arise from cells of the nucellus located mainly near the micropylar end of the embryo-sac. About the time of the first division of the fertilized egg, one or more nucellar cells may begin to divide, producing cell masses which grow into the embryo-sac and form embryos. In some forms most seeds are polyembryonic, and occasionally a mature seed may contain as many as ten or fifteen recognizable embryos. In such cases the embryos vary greatly in size, and the number capable of germination in one seed rarely exceeds three or four. Even within one species, varieties differ greatly in average number of nucellar embryos. Consequently, and also because the generative embryo is often eliminated as a result of competition or inherent weakness, the proportion of generative seedlings varies greatly. In some varieties less than 10 per cent. of the seeds produce generative seedlings. In fact, the seeds of some hybrids, such as the Sampson tangelo, apparently never produce any generative seedlings. It is possible that in some forms nucellar embryony is stimulated by pollen without the occurrence of fecundation, and even that, in exceptional cases, nucellar embryos may occasionally form in unpollinated pistils. A high proportion of nucellar seedlings tends to make a clonal variety unsuitable for use as seed parent in crossing; on the other hand, it is a great advantage in the production of seedlings for use as budding stocks, since the nucellar seedlings, because of their asexual origin from seed-parent cells, are normally identical in genetic type with their seed-parent.

Clonal Senescence.

A clonal variety of *Citrus* which is many years old from seed usually is thornless or nearly so. In most such varieties, any young clone, recently initiated by propagation from a young *nucellar* seedling, is extremely thorny. Such young clones also show much more vegetative vigour, and less tendency to blooming, than do trees of the old parental clone at the same age from budding. In the course of years, these juvenile characteristics gradually become less marked in the newer shoot growth and in the trees of successive propagations. Young clones derived from generative seedlings and ones from nucellar seedlings show, on the average, similar behaviour as to occurrence and decline of thorniness, and it is therefore concluded that sexual and asexual seed reproduction have essentially the same effect in producing seedling rejuvenation in *Citrus*.

Cytology.

Normal Chromosome Numbers.—In all species and varieties of *Citrus* yet examined, except for certain polyploid forms found in special breeding cultures, the somatic number of chromosomes is 18. The same is true of *Poncirus*, *Fortunella*, and four other genera colsely related to *Citrus* (*Citropsis*, *Severinia*, *Triphasia* and *Aeglopsis*), except that *Fortunella hindsii* (Champ.) Swingle has 36 chromosomes. The chromosomes are small, and

do not differ greatly in size. At the microspore-tetrad stage, supernumerary microspores (microcytes) are rather frequent in some varieties.

Polyploidy.—Under certain climatic conditions (frequently at Riverside, California), perhaps as a result of low night temperatures in the blooming season, various ordinary diploid (18-chromosome) varieties of *Citrus* produce occasional tetraploid (36-chromosome) nucellar seedlings, and when cross-pollinated such diploids occasionally produce triploid (27-chromosome) hybrids. Since a polyploid embryo is very often accompanied by one or more normal diploid embryos in the same seed, it is probable that this doubling of chromosome number usually occurs during the formation of the nucellus, or at most not earlier than the early stages of the development of the ovule. Triploids form trivalent chromosomes to a very large extent at the first meiotic division in the pollen mother cells, together with frequent bivalents and univalents; at the sporad (spore tetrad) stage the microspores are decidedly variable in size, and supernumerary microspores are abundant. Tetraploids form quadrivalent chromosomes to a large but highly variable extent in the pollen mother cells, and usually have much irregularity in meiotic chromosome distribution. In most tetraploid forms examined, the numbers of supernumerary microspores have been similar to those in triploids; in the Tetraploid (Owari ?) satsuma they have been decidedly less numerous, and in the Tetraploid Lisbon lemon the percentage of irregular sporads has been very small.

Sterility.—Many triploids, but not all, are unproductive of fruit; the fruits of triploids are seedless or very few seeded, presumably because few functional gametes are produced. Tetraploids also vary greatly in fruit production. Their seed production per fruit has commonly been lower than that in the corresponding diploid forms, in some varieties much lower. In several varieties, however, it has been similar to that in the diploid, and in the Tetraploid Lisbon lemon it has been much higher. In many diploid forms also, absence or scarcity of seeds or of *generative* seedlings is indicative of some form of sterility. Intergeneric hybrids seem to have a special tendency to sterility, but some such hybrids produce generative seedlings freely. In the ordinary seedless varieties of navel orange, the pollen mother cells degenerate without producing any mature pollen, and only a few of the ovules develop functional embryo-sacs. In the satsumas this sort of sterility is present but less marked; pollen is produced, but is seldom if ever functional. Many seedy diploid varieties have large proportions of visibly bad pollen. Some varieties are characterized by abortion of a great part of the pistils. In varieties producing good pollen, the pollen mother cells often degenerate without dividing in a large part of the flowers; such degeneration seems especially common in certain tetraploids which produce few seeds.

GENETICS.

The Limits of Crossing.—*Citrus* forms seem to be generally self-compatible and cross-compatible so far as they produce functional gametes, although some evidence tends to indicate the existence of rare exceptions. The crossing of *Citrus* with the most closely related genera seems more difficult to accomplish than crossing within the genus *Citrus*, but vigorous hybrids have been secured from *Poncirus* and from *Fortunella*, while weak or abnormal hybrids have been secured from *Murraya* and also from the crossing of *Poncirus* with *Fortunella*.

Heterosis.—With polyembryonic forms, the average vigour of generative seedlings seems to increase, in general, with the unlikeness of the parents, from selfing of *Citrus* to the crossing of *Citrus* with *Fortunella* or with *Poncirus*. Selfing, or the crossing of closely related varieties of the same species, such as varieties of sweet orange, commonly gives weak generative progeny. However, many of the crosses which have been made between varieties of the mandarin group have produced decidedly vigorous hybrids—a fact of possible significance in relation to the taxonomic problems of this highly diversified group, which has commonly been included in the single species *Citrus nobilis* Lour.

Variability and Heterozygosis.—In those varieries of *Citrus grandis* Osbeck known as the shaddocks or true pummelos (as distinguished from the grapefruits and intermediate forms), nucellar embryony seems to be entirely absent, and young seedlings of such varieties have been found to be comparatively uniform. On the other hand, the F_1 hybrid seedlings produced by intercrossing any two polyembryonic forms, even varieties such as the King mandarin (*C. nobilis* Lour.) and the Lisbon lemon (*C. limonia*

Osbeck), which have few seeds with more than one embryo are remarkably variable in tree and fruit characters; in fact, they suggest F_2 hybrids from a cross involving many gene differences. Great variation in many characters in each cross is the rule, as in vigour of growth, susceptibility to disease, size and shape of leaf and fruit, colour of rind, acidity and aroma of juice, number and shape of seeds, and amount of nucellar embryony. In a particular character, hybrids from one cross are most often more or less intermediate but often closely resemble one or the other parent, or even fall outside the parental range. Great variablity is the rule also among generative seedlings that result from selfing of any polyembryonic form. It is therefore probable that the *Citrus* forms which produce nucellar embryos are, in general, extremely heterozygous. This interpretation is favoured also by the abundant occurrence of degenerated pollen in these forms, and by the striking tendency to lack of vigour in generative seedlings from selfing or narrow crossing. Probably much of the partial sterility mentioned above results from segregation or homozygous combination of recessive genes unfavourable to gametophytic or zygotic development. In breeding for the production of superior horticultural varieties, the usual high variablity of generative seedlings is favourable to success with F_1 hybrids, and the vigour relations are comparatively unfavourable to success with selfing or with narrow crossing. Nucellar embryony by providing a natural means of asexual multiplication, has doubtless been very favourable to the perpetuation of heterozygosis, whether this has arisen through gene mutation or through hybridization or in both ways.

Individual Genes and Gene Mutation.—With polyembryonic forms at least, the variability is so great and so confused that positive identification of the effects of single gene differences is extremely difficult, if not entirely impossible. Even with such colour characters as the purple colour of the flowers and young leaves of lemons, or the red pulp colour of blood oranges, different F_1 hybrids from one cross may either develop the character in various degrees or lack it entirely. Production of general homozygosis by selfing is presumably impracticable, because of the tendency to weakness and sterility. One very striking character, the brachytic or shortened condition of the leaves and internodes in a dwarf form of sour orange,[1] seems likely to be due to one dominant gene, since in a cross with grapefruit the brachytic character appears in about half of the F_1 hybrids, the rest being ordinary in growth habit. Probably homozygosis for one recessive gene is often responsible for occurrence of dry pulp or for absence of chlorophyll, both in generative seedlings and (by somatic variation) in nucellar seedlings or in branches of normal trees. Among generative seedlings, the excessive segregational variation obviously makes the recognition of gene mutation extremely improbable. Gene mutation is suggested by various somatic variations, including "bud variations" and differences among nucellar seedlings of the same parent. It seems entirely impracticable to determine how far such changes in *Citrus* may be due to other causes, such as gene reduplication or somatic crossing-over, although the somewhat frequent occurrence of "twin" rind variations, producing opposite changes in adjoining sectors of rind, indicates that in many cases the cause is gene segregation rather than gene mutation. In several cases where nucellar seedlings are unlike their parent, there is strong evidence of very frequent genetic variation in the new characteristic, which suggests the presence of a highly "variable" or unstable gene. Some varieties of blood orange, such as the Ruby, have a mottled distribution of the red pulp colour which suggests a genetic cause similar to that which produces variegated pericarp colour in maize.

Polyploids.—Nucellar tetraploids show conspicuous differences from nucellar diploids of the same parentage. The leaves of tetraploids tend to be broader, thicker, and darker green; growth is slower, and tree size is consequently smaller and less erect; there is less tendency to bloom and set fruit, some tetraploid forms being very unproductive of fruit; the fruits have thicker rind, larger and often more prominent oil glands, stouter juice vesicles, and more fibrous internal structure. Hybrid triploids can be tentatively determined by their resemblance to tetraploids, especially in thickness of leaves and size of rind oil glands, together with their scarcity of seeds

[1] This has been identified as the form known in France as "Bouquet de Fleurs", and is at least very similar to the form called "Bigaradier Riche Dépouille" by Risso and Poiteau.

and often of fruits. The general variability of generative seedlings, however, makes positive determination of triploids difficult or impossible without examination of the chromosomes; the condition of the microsporads, which are much more easily obtained and examined than are adequate chromosome figures, seems to be nearly conclusive as a rule, since tetraploids have not been found among generative seedlings. Pollination of a tetraploid grapefruit form by diploid varieties has given a fair number of seedlings, including a few hybrids; as was to be expected, pollination of diploids by this tetraploid was unsuccessful. Triploid hybrids have been produced by crosses between diploid *Citrus* and the tetraploid *Fortunella hindsii*.

Chimeras and Bud Variation.—Chimeras of graftage origin, which exhibit typical characters of two species in different parts of the same tree, and are largely intermediate between those species, have been known for nearly three hundred years under the name of "bizzarria". These evidently are fundamentally periclinal chimeras, with frequent secondary sectorial or mericlinal conditions. Certain green-and-white forms, some of which are known to have originated from ordinary varieties by "bud" (somatic) variation in orchard trees, are obviously also periclinal chimeras, with much irregular sectorial arrangement of the two types in the rind of the fruit. These can usually be classified readily as fundamentally white-over-green or green-over-white periclinals; the former usually show much more non-green and light or pale-green leaf tissue, but both are extremely variable as to presence, size, and arrangement of the differently coloured regions. The non-green regions of rind may differ from the normal rind in other characters also, such as thickness, gland characteristics, and development of orange or yellow colour at maturity. Other bud-variation forms have normally green leaves, but show sectorial rind conditions that indicate the presence of a variant genetic type which probably constitutes with the normal parental type a periclinal chimera. In such forms the amount of rind variation probably depends largely on the relative growth vigor of the two component genetic types, and their relative position as "core" and "skin" in the apical meristem, as well as on the numbers of meristematic layers of the components. A bud variation shown by a branch or a fruit obviously may be due to a recent genetic change in a meristematic cell, or it may be due to accidental emergence of a type long present as the inner component of a chimera. The frequent occurrence of bud variation in polyembryonic *Citrus* varieties is presumably due fundamentally to gene shifting (crossing-over, reduplication, etc.) and possibly gene mutation in extremely heterozygous chromosome complements, which readily permit such changes to produce perceptible somatic effects. Therefore it is probable, in view of the preceding discussion of variability among generative seedlings, that nucellar embryony, by favouring the evolution of excessive heterozygosis, has been a dominant agency in establishing the remarkable generative and somatic variability of *Citrus*.

Limitation of space prevents citation of the numerous papers on which this summary is based. The reader is especially referred to the following publications, and to the bibliographies therein given:—

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PHYLOGENY IN THE LIGHT OF GENETICS AND CYTOLOGY.

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(Received 24th August 1936.)

PHYLOGENY, or the genealogy of extinct and living organisms, is an inevitable consequence of organic evolution. In this broad sense, phylogeny is necessarily a highly speculative phase of biology and it is likely to remain so indefinitely for the simple reason that extinct animals and plants cannot be subjected to experimental research. Nevertheless, the conception, that existing organisms can be classified according to their phylogenetic relations or order of occurrence in the vast network or tapestry woven by evolution, has been of very great service to biology. Thereby, the basic science of taxonomy has become increasingly scientific through closer approximation to the truth, even though there still exists among taxonomists the greatest diversity in the conceptions held as to the nature of the basic taxonomic unit, the species. A comparison of the systems of classification adopted by Linnaeus and his predecessors with those in vogue to-day shows that modern taxonomy has advanced beyond that of the eighteenth century very far towards the goal of a natural system of classification.

The progress thus far made towards this goal has been accomplished largely through more critical studies of comparative morphology, supplemented by the evidence from comparative anatomy and histology and by the study of geographic distribution. The growing recognition of species as groups of individuals and the increasing use of geographic distribution as one criterion of relationship have induced taxonomists to examine more individuals before making inferences about the limits of species. In this connection the use of statistical methods has become more general, and the inferences concerning the significance of individual differences are thereby usually more dependable. In all this progress the important guiding principle has been the dynamic Darwinian concept that existing species have been derived from pre-existing species.

Although comparative morphology, anatomy and histology are important criteria of phyletic relations, yet their value will always be limited by the nature of the data which they provide as a basis for inference. These data are obtained by the method of observation, and by this method alone, it is impossible to test the validity of the infereences drawn, except by making further observations. It is for this reason that taxonomy is beginning to develop into an experimental science. The leaders in this movement foresee a new taxonomy which will combine traditional methods with experimental ecology, genetics and cytology. Synthesis of the evidence from both observation and experiment should reveal the true significance of the resemblances and differences between individual organisms and, hence, should lead to sounder taxonomic, including phylogenetic, concepts.

Genetics and cytology provide only part of the evidence upon which phylogenetic conclusions must be based, but the nature of this evidence makes it highly valuable. Since genetics deals with the inheritance of all the structures and functions of organisms, comparative genetics of related species and genera should provide direct evidence on the phyletic relations of such groups. But the genetic analysis of species involves extensive, time-consuming research, and the comparison of the genetic characters of different species by combining them in interspecific hybrids is often difficult or impossible in plants as well as in animals. Therefore, while genetics is of great value to the experimental taxonomist in solving problems which require genetical data for their solution, genetic analysis alone does not hold great promise as a means of rapid progress in testing phylogenetic hypotheses. Cytology, on the other hand, deals largely with the carriers of the hereditary units or genes, the chromosomes, and data on the chromosomes are readily obtainable from the living organisms. Furthermore, methods have recently been devised by which the chromosome relations between plants represented only by herbarium specimens can be ascertained with a high degree of dependability, provided that some comparable material is available for cytological study. This facilitates considerably the working out of phylogenetic series. Also, genetics has been combined with cytology in research on both interspecific and intergeneric hybrids

among the flowering plants with the result that considerable light has been thrown on the phyletic relations of the parents.

Chromosome Number.

Difference in chromosome number among the species of a genus is a phenomenon of widespread occurrence among both animals and plants. But differences in chromosome number are of various sorts and have diverse significance. For example, in *Crepis*, a large genus of the Compositæ family, there is a partly consecutive series of haploid chromosome numbers, as follows: 3, 4, 5, 6, 7, 8, 11, 20, 22, 44. From this series alone, there is no way of predicting which, if any, is the most primitive number. Of course the five higher numbers could have been derived from some lower number or numbers. But among the latter, which is most primitive? The statistics on comparative numbers of species in the genus having the different chromosome numbers have been thought by some to answer this question. Because a large proportion of the species have four pairs of chromosomes, it has been inferred that four is the primitive number. But by combining the evidence on chromosome number with the evidence on chromosome morphology along with comparative morphology of the plants and geographic distribution, it has been possible to show (1) that 5 must be the primitive number in *Crepis*; (2) that all the 4-paired species were derived from 5-paired ancestors; and (3) that all the other numbers were derived directly or indirectly from 5. But there are many genera of plants in which all the species of a genus have the same chromosome number. Yet these species may differ widely and fall into diverse subgeneric groups. Furthermore, a few species have been examined that have variable chromosome numbers within a single species. Hence, we must conclude that chromosome number alone is not a sufficient basis for making definite inferences as to degree of relationship between species. When used in connection with other criteria, however, it may sometimes prove highly valuable.

Chromosome Morphology.

The size and shape of the chromosomes are, in many cases, more useful criteria of relationship than chromosome number. To illustrate again from *Crepis*, all the 5-paired species have distinct types of chromosomes, one pair of each, whereas all the 4-paired species lack a particular one of these types (with a single exception; a recently examined species, *C. oporinoides*, lacks a different member of the basic set of 5). With only these facts in mind, one might think it just as likely that 5-paired species were derived from 4-paired ancestors by addition of one pair of chromosomes as that 4-paired species came from 5-paired ancestors by processes involving the disappearance of a pair of chromosomes. But aside from the technical details involved (and their consideration favours the latter view), when the evidence from comparative morphology is considered along with the cytological evidence, it is clear that 5 is the more primitive number, for the species of *Crepis* which are most primitive morphologically have 5 pairs of chromosomes. We may emphasize, therefore, that in the study of relationships between species, the evidence on number and morphology of the chromosomes should be used in connection with all other available evidence. Only in this way can sound conclusions be reached.

Chromosome Behaviour in Meiosis.

This is a very large subject and space permits only a few general statements which are intended to show how this type of evidence bears on phylogeny.

The regular appearance of paired homologues at diakinesis just preceding the first meiotic metaphase is characteristic of normal diploid species. The process of pairing has been observed in the early prophase of this division and it is known to occur chromomere by chromomere. Hence, it is inferred that there is some sort of attraction between homologous genes during that phase of meiosis. If regularity of pairing is correlated with gene homology, then the degree of regularity of pairing should be an index of the purity and constancy of a species and a useful criterion of relationship between species. (Reservations must be made in this connection covering asynaptic genes and certain polyploids.) Interspecific hybrids usually exhibit more or less irregularity in the formation of bivalents at diakinesis. The degree of irregularity displayed by such a hybrid is to some extent an index of the degree of homology existing between the chromosomes of the parent species and hence of the degree of relationship of those species. Yet the occurence of pairing in an interspecific hybrid is not, in itself, a reliable criterion of relationship. Degree of fertility in such hybrids is sometimes correlated with irregularity of pairing

and distribution of the chromosomes in meiosis. But caution is also necessary in using fertility of hybrids as a criterion of relationship between the parents.

In short, the cytogenetics of interspecific hybrids is a rapidly developing field of biology. Important discoveries are being made which bear directly upon phyletic relations. But the utilization of this evidence by taxonomists will require considerable attention to the technical aspects of the subject-matter. The same may be said concerning other newly developing phases of cytogenetics, such as the structure of the salivary gland chromosomes in insects.

Modern taxonomy is based on evolution as a guiding principle. One of its chief aims is to discover phyletic relations and establish lines of descent. Comparative data on the chromosomes of species and critical study of the distribution of the chromosomes during the gametogenesis in hybrids furnish valuable criteria of relationship between species. But, in order to make conclusions regarding phylogeny as reliable as possible, it is necessary to use all available criteria and to synthesize all the evidence.

ADAPTATION IN THE LIGHT OF GENETICS.

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(*Received 31st August 1936.*)

MODERN genetics indicates that the bulk of evolution in the higher organisms has consisted of (1) gene mutations or other chromosomal modification, (2) recombination through biparental reproduction, and (3) preservation and elimination of certain lines of descent. Have these processes also led to the obvious and widespread fitness of living things to their environment?

The principal conceivable means of attaining fitness independently of evolution is by migration of organisms to the situations in which their structure and physiology will work best. There are reasons to think that a certain amount of favourable adjustment has been produced in that way. Goldschmidt finds races of the gypsy moth fitted by their speed of development to regions of different climate, and conceives that they have been lucky enough to find the suitable places. Moreover, many features of the environment, such as temperature, or salinity of water in estuaries, display a gradual change from one extreme to another, and it should not be difficult for organisms already in possession of their qualities to move slowly along such a gradient to the most favourable point. Adaptation is, however, so prevalent, and at times so specific or so isolated, that migration seems inadequate to account for it. There must be some way of producing fitness during and as a part of the evolution process.

It is conceivable that adaptation might result directly from the initial changes of organisms. Were there some way of guiding gene mutations or chromosome rearrangements into useful channels, fitness would have a very simple explanation. The simplicity and directness of this scheme has led many in the past, some even now, to adopt it. The Lamarckians, if they subscribe to the general method of evolution outlined above, must hold that the soma exerts such a directive influence on the mutations occurring in the germ-cells within it. It is the testimony of genetics that such an influence does not exist, or at most is unimportant. Yet some comfort has been given to Lamarckians by the recent artificial production of mutations by X-rays and heat. Certain of these artificial mutations have arisen in generations later than the one treated, indicating some storage of the environmental effect, perhaps by the cytoplasm. Could such storage be counted on to apply to all sorts of external influences, genetic change might be supposed to result eventually even when prolonged experiments fail to reveal it. None of the delayed mutations so far obtained, however, gives any more indication of being useful than do other mutations, so that the probability that adaptation has a Lamarckian origin is no greater than formerly.

Mutations occurring naturally in the many organisms in which they have been observed likewise show no sign of adaptiveness. As a rule they are demonstrably harmful, though some of them are approximately neutral. Occasionally, as is true of the vermilion eye mutation of *Drosophila hydei*, the mutant gene can, at least for a considerable time, hold its own beside the older gene in natural populations. On the whole, however, it must be recognized that the mutations *observed* are not useful ones.

Whether the same conclusion must be adopted with regard to physiological mutations, which are seldom discovered but which are probably more important to the organism than are the structural changes which experimenters detect, is a question for which there is no answer. Since, however, the nature of a mutation must be determined primarily by the chemical structure of the gene that is changed, fitness to the environment would hardly be expected as an immediate consequence.

If gene mutations do not directly provide adaptation, what may be said of the second factor of evolution as outlined in the opening paragraph, namely, recombinations? Recombinations, once a mating is made, appear to occur at random. The only guidance which could lead directly to adaptation would therefore necessarily apply to the choice of mates. In the evolution of domestic animals and plants such guidance is furnished by the breeder. In nature, however, there is nothing to replace the breeder in this particular part of his activities. Though some animals mate and others do not, their choices appear to have no relation to the usefulness of the consequences. This statement ignores sexual

selection, which is to be mentioned later; it is not intended to answer the question whether secondary sex characters, particularly ornaments, are useful.

Preservation and elimination of lines of descent are the remaining means to adaptation. Fortunately, they appear to be adequate to the task. Natural selection, against which the genetics of a third of a century ago was then thought by many eminent biologists to speak strongly, is regarded as a necessary corollary of the genetics of to-day. Genes tend to remain for ever in the same numerical relation to one another, except as that relation is disturbed by accident, by mutation, by migration, or by the value of the characters determined by them. The calculations of Wright, Fisher and Haldane have shown what is to be expected from these several sources of change, singly and in various combinations, in populations of different sizes, and the value of the characters is found to be by far the most powerful. Useful genes tend to be preserved and to increase in frequency, harmful ones tend to disappear. The present concept of what constitutes usefulness has been furnished, not by genetics, but by a more careful mathematical consideration. Qualities to be useful in an evolutionary sense must lead to more descendants. A gene which insures its possessor more descendants than its alternate genes do, is bound to occur in increasing numbers of individuals and eventually replaces its alternates.

At what point in the history of a gene natural selection commences to affect it, depends partly on whether the gene is recessive. Fisher, holding that a new gene is seldom if ever wholly recessive, conceives that selection starts at once to favour or oppose it. Wright, on the contrary, holds that accident is largely responsible for a gene's early fortunes, and that varietal or even specific differences may arise largely through chance. The latter view is more in keeping with the fact that most varietal and specific distinctions appear to have no selective value. Wright points out that a large species broken up into many local races furnishes the best opportunity for rapid evolution.

How evolution that is adaptive can arise out of mutations which are harmful or at best neutral has puzzled many. It has seemed to them that there must be mutations which we do not detect, perhaps mutations of a different sort, which are adaptive at the outset. Doubtless there is mutation of this advantageous kind, quite possibly more of it than anyone surmises. To most geneticists, however, the detecting of almost solely harmful mutations, and the passing over of enough useful ones to account for the great amount of adaptation there is, have seemed to require contradictory powers of observation. A way out has been suggested by Wright, who takes advantage of one of the common consequences of recombination, namely, enormous variety in gene interaction. Probably every gene has several effects, depending on what other genes are present. Hence, a gene that is harmful among the genes with which it is first associated may, through recombination, become associated with others to which its response is beneficial. Examples of great difference in value in different combinations have already been found. A gene may thus survive, even though harmful, because it is recessive and because of its good luck, and so enter into many combinations. Eventually a valuable combination may occur, and then the gene comes to be positively favoured. Inasmuch as adaptation is not universal, this appears to be at least a possible method of attaining it in the end.

Had the present knowledge of genetics been available seventy years ago, the literature of evolution must have been very different from that which our libraries now contain. It is to be hoped that future literature of evolution will not suffer from the same deficiency. It is scarcely advisable to go on speculating concerning the course of evolution in situations where the genetic facts might be but have not been obtained. Comparative anatomists must still presumably base their evolutionary conclusions on logic, since their body of facts is derived usually from organisms too distant from one another in the taxonomic scheme to permit hybridization. But in all instances where the evolution under scrutiny has resulted in different forms still capable of entering into fertile crosses, there appears to be no justification for failure to seek the genetic facts.

Among the supposed adaptations which constitute differences between organisms still capable of hybridization must be a considerable number of those involving animal colouration. The different types of conceivable colour adaptations differ considerably in their amenability to the genetic mode of attack. In protective resemblance, it would be necessary to have, besides the protectively coloured species, one of its close relatives which is not protectively

coloured or which is adapted to some other background. A knowledge of the number of genes in which they differ, and how these genes are transmitted, would tell a great deal about the probable mode of their separation. Quite possibly this information is unobtainable in most instances of supposed protective resemblance, but there is little to indicate that it has been sought.

Sexual selection offers some difficulties also, because the presumed adaptation exists in only one sex. Production of secondary sexual characters in vertebrate animals is dependent largely on hormones and some genetic, and a great deal of physiological work is fortunately being done in the general field of endocrinology. Time may well bring forth the genetic knowledge that is required in this group. There appears to be room, however, for much more genetic work on secondary sex characters in other groups, particularly insects.

Greater opportunities for pertinent genetic studies and quick results that are significant appear to lie in the realm of warning colour and mimicry—the latter more than the former. Supposed warning colour often exists in whole groups of animals so that no near relative is available for crossing. In mimicry, however, there should be no such obstacle, for one of the prime reasons for suspecting an evolution guided by the model is that the mimic is strikingly different from its near relatives. While not all of these near relatives are likely to be crossable with the mimic, a percentage of them should be. This particular type of adaptation should, therefore, be capable of genetic study.

Fortunately, some genetic work has been done on mimics. Part of it has consisted in bringing females into the laboratory after mating with unknown males, and observing their offspring. Heterozygosis of one parent or the other has thus been discovered. A few experiments have been carried out for the express purpose of learning the probable origin of mimicry. It is this type of investigation of adaptation that is to be particularly commended.

The general results of such studies of insect colouration have been that colour modifications have been fairly simple. Mimics have been found to differ from their relatives in one or two pairs of genes. Should other adaptations prove to be as simple, our conception of their origin should be profoundly modified. The general concept of such colour adaptations has been that they started with slight fortuitous changes, that other similar changes were added to them and preserved because they were advantageous, so that the present colour was attained gradually over a long period of time. This concept is untenable for a character arising through only one or two mutations. If similarity to another, not closely related, species is attained at one step, the supposed usefulness of such similarity has nothing to do with its origin. The advantage of the resemblance, if there be any advantage, may help to preserve the mimicking form; but this preservation had nothing to do with making the change useful. Under these circumstances a specific difference which makes one species look like another unrelated to it is no more likely to be useful than any other specific differance is. Since most specific characters are not useful, resemblance to an unrelated species is likely to be useless, if it arises by one mutation, or even by two. It is important, therefore, to know the genetics of resembling but not closely related species before it can be decided whether the similarity has any evolutionary significance.

Another feature of colour which is of evolutionary importance is pattern. This was long regarded as an embryologist's problem, not a geneticist's; but it is the sort of thing that future geneticists will be most concerned with, namely, the mechanism of development. Some of the alleged colour adaptations involve similarities of pattern as well as of colour, and this has led many to conclude that the likeness must have originated under the guidance of some organism's visual powers. If we knew how members of the *same* species come to have the same pattern, we should understand better why *unrelated* species sometimes have similar patterns. Some principle of development is involved. If the mechanism is capable of working in a limited number of grooves, it ought not be impossible for the same grooves, or similar grooves, to exist in insects belonging even to different subfamilies.

In conclusion, it may be said that the little available genetic evidence that bears on adaptation indicates that some of the qualities regarded as adaptive probably are not so; and that those which are adaptive may easily have risen in a much more direct manner than that which has long been postulated.

GENETICS OF HUMAN INTER-RACIAL HYBRIDS.

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(Received 29th June 1936.)

THE phrase " inter-racial hybrids " is one borrowed from animal or plant breeding where more or less pure bred races exist. Among humans, however, pure bred races are not so common and are rapidly becoming less common on account of the improvement in means of transportation which has led to a rapid intermingling of such races as had been evolved in the preceding centuries. Hence it is that the study of genetics of inter-racial hybrids in man is difficult, and becoming more so. However, in the midst of all this intermingling, the human racial traits, such as those of eye colour, form of hair and facial features, skin colour and many others, often remain intact, so that a study can be made of the genetics of crosses between different race traits.

Human hybridization is, as stated, occurring very rapidly and has been taking place for generations, in fact, no doubt, for hundreds of years. When David Livingston first crossed Africa he found Jews and Arabs already on the west coast, where they had been carrying on trade for generations, and their presence was accompanied by hybridization. Similarly, hybridization, on a vast scale, has taken place between European stocks on the one hand and Asiatic stocks in North Africa and the peoples (blacks) of middle Africa on the other. Such hybridization occurs in the Americas because of the earlier importation of Negro slaves. Similar hybrids have occurred in great number between Europeans and American Indians, between Europeans and Asiatics, between Asiatics and Malays and other peoples of Melanesia and Polynesia. Thus there would seem to be abundant material for study of inter-racial hybrids, but unfortunately there are usually no records and we are usually not in a position to study the consequences of the first and second generations which are crucial for genetical interpretation.

That it is important to determine the consequences of inter-racial hybridization need hardly be argued. The hypothesis is extant that some inter-racial human crossings (like that of some dogs) leads to physically or mentally abnormal individuals. In view of the fact that inter-racial hybridization has taken place on so great a scale mankind ought to be informed of the truth or error of this hypothesis.

The fundamental studies that have been made upon the inter-racial hybrids are not very numerous. Mention may be made to Eugen Fischer's *Die Rehobother Bastards*, to Rodenwaldt's *Die Mestizen auf Kisar*, and the studies of Davenport-Steggerda, Danielson, Herskovits and others, on crosses between Whites and Negroes. Dunn has also published on data collected in the Hawaiian Islands on crosses between Europeans and Polynesians.

The genetical results of studies in such inter-racial hybrids may be grouped under two heads, the first referring to physical traits and the second to mental traits.

That races differ in physical traits no one can deny. Matters of skin colour, of facial features, of hair form and colour, eye colour, head form, stature, are too obvious to be doubted. Some of these physical traits are inherited in simple Mendelian fashion, others in more complex fashion and, therefore, cannot readily be reduced to Mendelian principles. A few of the results of studies of inheritance of particular traits are added.

Eye colour occurs in two principal forms, brown or melanic pigmentation, and absence of such giving rise to clear blue eyes. The absence of the brown pigment seems to be a mutation that occurred in North-Western Europe and has been carried by migration to different parts of the globe. The presence of brown pigment in the Irish is dominant over its absence. These results were worked out by C. C. Hurst and the Davenports.

Skin colour is inherited in more complex fashion. In crosses between brunettes and blonds the offspring show an intermediate degree of pigmentation. In crosses between Negroes and Whites the skin colour is also intermediate. This cross has been studied in detail by Davenport and Danielson, and the conclusions drawn that there are two pairs of factors for skin colour in the full-blooded Negro, that the mulatto has two of them, the quadroon has phenotypically one, and the so-called sambo has phenotypically three.

The results of study on hair form in hybrids has been worked upon by Fischer, Davenport and Danielson, Davenport and Steggerda, and Bean. Fischer, in 1913, among the Rehobother Bastards found woolly hair in 29%, frizzy to wavy hair in 49%, straight hair in 22%. In Jamaica, whereas 85% of the full-blooded blacks had the diameter of the curl of the hair under 5 mm., no whites have any such hair. Of the browns over 50% had hair with such a close coil, but in the browns there was 90% of hair with diameter of curl of 9 mm., or less, as contrasted with 100% of the blacks and 2% of the whites. The conclusion was drawn that curly hair is dominant over straight European hair, but not completely so. Bean, on the other hand, found that in crosses of Chinese and Philippinos coarse black hair of the former tended to dominate over wavy hair of the latter.

Form of the head varies in races of mankind from long to short. This has been made the subject of special study of Frets, and he has concluded that brachycephaly is generally dominant to dolichocephaly. There are, however, various size factors concerned in inheritance of head shape so that its genetics is not easily determined.

Inheritance of stature has been studied by the old statistical school and, subsequently, by the writer, on a Mendelian basis. It appears that while there are two, or more, factors involved, the shortening factors are generally dominant over those responsible for great stature.

Races differ in their types of blood from those which have prevailingly the A agglutinating factor, the B factor, and no agglutinating factor, of which the Amerindians seem to be an example. The A or B agglutinating factor is dominant over the absence of either factor, but the A and B factors are regarded as two allelomorphs occupying the same locus.

The existence of a genetical basis for differences in mental traits in different races of mankind has been vigorously denied, especially by Franz Boas and his school or, at least, they have insisted that there is no proof of any such difference. Attempt to get light upon this subject was made in the studies of Davenport and Steggerda, who applied different types of mental tests to whites and blacks from Jamaica and the Grand Cayman Islands. It was found that in musical capacities, in general, the blacks stand first and the whites last. In the drawing of a man the whites excelled. In fitting blocks into mortised spaces and in the form substitution test the whites worked faster and more accurately than the coloured persons. Also, in the so-called cube tests the whites were clearly superior. In the repetition of seven figures the blacks stood first, but in the criticism of absurd sentences the whites were superior. The psychological testing also showed that a small proportion of the hybrid browns *did worse* than either the blacks or the whites, suggesting that hybrids are not only more variable, but some of them show a mental state of excitement, or confusion, which resulted in complete failure to do the test.

While studies on the genetics of interracial hybrids among humans, or genetics of human hybrids, have been made in greatest volume, perhaps the most analytical work is that done on dogs by Dr. C. R. Stockard. He has crossed races of dogs differing widely in form and instincts and reached important and striking results. These studies reveal that the differences between the races of dogs may be, in part, due to difference in endocrine activity; but that in addition to that factor there are direct growth-promoting factors that differ. His experiments demonstrate that a physical disharmony may arise in crosses of dissimilar races. For example, in the cross of a Boston terrier and a Dachshund, in the second generation, he obtained long muzzles and upper jaw associated with the Boston short lower jaw, making it difficult for the hybrids to feed. Again, in crossing long-legged with short-legged achondropastic types, the short legs may be inherited in the anterior appendages and not in the posterior appendages so that the hybrids move with considerable difficulty.

The interesting results of the work on dogs thus supports the view of hybrid disharmony, and makes more probable the interpretations that have been made on crosses between human races (as for instance Whites and Negroes) which seem to point to occasional marked disharmonies in the hybrids so that some hybrid individuals are inferior in performance to any individual in either of the constituent races.

In view of the great importance of a precise knowledge of genetics in interracial hybrids, it becomes of value to secure studies in regions where hybridization is

just beginning so that the first and second hybrid generations may be definitely known. There are a few regions on the earth's surface where such studies can be made. One is said to be the Amoor River region in Siberia, where the Siberians are coming in contact with the Japanese. There must be regions in Africa where the Whites are coming in close contact with practically full-blooded Negro stock. There are still regions where full-blooded Amerindians are coming in contact for the first time with Whites. The same is true of the Eskimo of North America. It is to be hoped that before it becomes too late funds will be provided by means of which such fundamental studies of consequence of human hybridization may be made. At the same time great contributions will be made to our knowledge of inheritance of human traits.

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THE FUTURE OF GENETICS.

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(*Received 7th November 1937.*)

IN regard to the invitation of the Editor of *Current Science* to contribute an article on the future of genetics, I must confess that I accepted with trepidation. Such a discussion has little margin between emphasizing the obvious on the one hand and venturing into inspirational prophecy on the other. The best that can be done legitimately is to examine the present trends and extrapolate toward expected goals.

From the extrapolation method we may expect advances on the following broad fronts: (1) an increasing range of plant and animal forms where the genetic situation has been explored and tied in with the already established phenomena and laws of heredity; (2) the intensive study of forms that have failed to conform to established principles; (3) with the consequent extension of those principles and the invention of new hypotheses or simplification of the old; (4) the discovery of new technical methods in breeding tests, cytological checks, and treatments by various physical or chemical agencies and environmental factors; (5) a widening interaction with parental sciences such as cytology and chemistry, with sibling sciences such as embryology and physiology and with offspring sciences such as eugenics and the improvement in genetic type of plants and animals useful to man; and finally (6) progressive clarification of man's philosophical outlook as to his origin, development and activities, with the rescue of still other departments of life from the deistic and vitalistic to the naturalistic and mechanistic realms. A few of the particular advances expected on some of these fronts will be discussed:—

The past three decades have shown that all the higher plants and animals have their inheritance based on the Mendelian principles of discrete representative determiners or "genes," which grow, reproduce themselves "autocatalytically," and undergo non-contaminative segregation and are distributed to individuals of the next generation through the chromosomes which synapse, cross-over and assort into the germ cells. It may be predicted that all forms that have evolved to the stage of multichromosomal nucleated cells will be found to have this astonishingly uniform pattern of inheritance —the same in man as in the fly.

In fungi, such as Neurospora, and in the forms with well-developed haploid generations the complication of dominance is removed and the simple situation will reward intensive study of gene action and sex. Especially noteworthy in Neurospora and such types is the fact that all the products of each reduction division are available for analysis as to the details of basic processes like crossingover.

In the Protozoa another type of complication, that of cell interaction during a long embryological development, is removed. In the still lower forms, where the organization may correspond to a unichromosomal of multigenic type, and in the very lowest forms, which may correspond to single genes, the most fundamental phenomena and those only are left for study. Studies of bacteria and viruses and phages are only beginnings but already promise brilliant illumination in the future.

The most fundamental problems of life seem to be epitomized in the growth and reproduction of the gene—the most basic unit thus far established, occupying for us the place held by the cell for a previous generation of biologists. Here we must analyse the gene in terms of physical structure and chemical behaviour. A promising lead pictures the gene as a crystalline body, probably of the fibre type, whose autocatalytic growth is by surface condensation of constituent simpler materials from the surrounding medium. The identity of the new growth with that of the original gene substance is governed by the specificity of the crystal surfaces, just as it is for alum crystals growing in a mother liquor. The reproduction of the gene is pictured as a simple split of this fibrous crystal when its growth has exceeded the size limits proper to its internal bonding *versus* the disruption due to temperature and other environmental factors, such as pH changes.

A second basic problem is the relation between the gene and the characteristics

displayed by the adult whole organism. This problem is the more complex as we pass upward in organization from a hypothetical simple ancestral gene to cellular and on to multicellular organ-differentiated types. We must look for our answer primarily in the direct action of the gene, by virtue of its chemical composition and the possession of catalytic surfaces, upon the materials immediately in contact with it. Chainwise processes must then be found linking these initial actions with changes in composition and properties of the materials of the chromosome, nucleus cytoplasm and circulating media of the body. Other chainwise processes must be found linking the structure of the fertilized egg with successive stages of differentiation during embryological development. The early elaboration of glands which specialize in producing growth- and reaction-controlling chemicals, the hormones, opens out for us the main lines along which our analysis will proceed.

Our model of gene structure must allow not only of autocatalytic growth and self-reproduction, but also of compositional change and the subsequent perpetuation of that new structure, *i.e.*, the origin and inheritance of mutations. On the hypothesis that the fields of force on the surface of the gene control its crystalline growth, it is conceivable that rare acceptance of a misfit building block would alter the surface material. Then the corresponding alteration of the surface forces would give the basis whereby such blocks would thereafter become the normal structure at that point, and the mutant would perpetuate itself.

Not only changes in the constitution (and hence properties) of a gene but changes in its position in the series of its fellows give rise to inherited characters. "Position effects" are well established as facts, and the main line of their interpretation seems likely to follow the idea that the action of a gene is limited by what materials diffuse to it. Hence if its accustomed neighbours are no longer supplying their elaborated products within diffusion range or in the normal concentration, then its chemical effect is perforce altered.

The use of radiations, and treatments by chemical agents, to cause revealing changes in the gene and the chromosome is only well begun, after some ten years of intensive application. By their physical and chemical attacks problems of the size, number, structure, mutability and action of the gene are soluble directly, or indirectly through study of rearrangements, deficiencies and duplications that arise from such treatments.

Cytology will make its most valuable immediate contribution in the continuance of studies of the salivary gland chromosomes. For the first time, the internal structure of a chromosome can be seen in enough detail to offer hope of relating the gene to a specified structural entity—though what that relation is will demand the best analytic attack for some years.

Several cytological 5-year-plans will profitably be devoted to study of the phenomena involved in the synapsis, crossing-over and distribution of chromosomes to the gametes.

The classical lines of work based on linkage studies will continue to throw important light on the interaction of adjacent and neighbouring genes in producing joint effects, on the difference in phenotypic characters affected by adjacent genes, and on the general distribution of the genes affecting a given characteristic. Part of this can be deduced directly from the linkage maps as they become richer in mutant loci and more accurate in the localizations. Part will be deduced from studies of effects produced when new juxtapositions and serial orders arise through inversions and translocations.

The study of the relation between linkage maps and chromosome structures, both of standard metaphase chromosomes and of the salivary chromosomes, will demand as a preliminary the making of much more accurate linkage maps and morphological maps of the visible chromosome detail. Numerous identity points in the two kinds of maps must be established by linkage studies and cytological observations on the break-points and limits of given deficiencies, inversions and translocations. After such accurate correspondence points have become close enough in their spacing, then the other relations can be formulated. One is the local or chromosomal "coefficient of crossing-over," which expresses for a given section the relative liability of the chromosome framework or intergenic bands to loosen their old joinings and reassemble with exchange of partners. The other is the "coefficient of mutation," which expresses

the relative stability or tendency to mutate of a given local stretch of genes.

After these extrapolations towards the "expected" one must point out that it is the "unexpected" that is the seed of the most fruitful developments—through the discovery of a new favourable experimental material, a new method of attack, or a new principle or working hypothesis. Among such "mutations" in the evolution of genetics may be mentioned the use of Drosophila as test material, the application of X-rays as a method of studying mutations, and the working hypothesis that genes are "autocatalytic catalysts". We may recall that when the breeding of Drosophila had been under way some five years astonishment was often expressed that there was still much being found out about the heredity of this tiny fly, and it was not anticipated that twenty years later nearly 300 investigators would be at work on Drosophila and still making rich discoveries. Nor did anyone suppose that Drosophila would be transformed from a nearly hopeless cytological object into one of the most favourable known—through study of the large chromosomes of the salivary gland cells. The success of the use of X-rays in producing mutations was not foreseen from the scanty results of the first trials of that method. Thus we may say that in the future it will be necessary and ultimately profitable to make numerous bold excursions into far-off territory. Many of the pioneers will end their days ingloriously or wander in the wilderness in time-wasting pursuit of a will-o-the-wisp. But from the bravery and perseverance of that group will come the new openings to fresh life for all.

1874-37.—Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent, and Published by
B. N. Sastri, M.Sc., A.I.C., A.I.I.Sc., for the "Current Science" Board of Editors, Indian Institute of Science, Hebbal Post, Bangalore.

Current Science

A MONTHLY JOURNAL DEVOTED TO SCIENCE

Published with the editorial co-operation of

ANNUAL SUBSCRIPTION Rs. 6. Foreign 12 sh.
Single Copy 12 as.

All Communications to be addressed to THE SECRETARY,
"Current Science", Hebbal P. O., Bangalore.